The British Mycological Society

196

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TRANSACTIONS

Volume XXXI

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Edited by
B. Barnes and W. C. Moore

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SOME NEW *SPHACELOMA* DISEASES OF ECONOMIC PLANTS IN MYSORE

By M. J. THIRUMALACHAR
Department of Botany, Central College, Bangalore, India

(With 9 Text-figures)

Species of the genus *Sphaceloma* de Bary, with their ascigerous stage *Elsinoe* Racib., have received a good deal of attention from plant pathologists on account of the scab or anthracnose disease they produce on plants of economic importance. Recent monographic studies by Dr A. A. Bitancourt and Dr A. E. Jenkins have greatly advanced our knowledge of the group. In India, only two *Sphaceloma* stages are known: one is *S. Fawcettii* Jenkins, which is the conidial stage of *Elsinoe Fawcettii* Bitan. & Jenkins, recorded on species of *Citrus* (Jenkins & Fawcett, 1933) and on *Hesperethusa crenulata*, from Bengal (Jenkins, 1936); the other is the *Sphaceloma* stage of *Elsinoe Bitancourtiana* Thirumalachar, recently described by me (1946). In collections made in Mysore, four more species of *Sphaceloma* were found, ome of the hosts being plants of economic importance. Further studies are being made to investigate the possible existence of the ascigerous stages. An account of the *Sphaceloma* stages is presented here.

(1) A *SPHACELOMA* DISEASE OF *SANTALUM ALBUM* L.

Santalum album, the sandal tree, is of great economic importance on account of the valuable essential oil contained in the heart wood. Diseases of the andal tree have been the subject of several detailed investigations in India. A species of *Sphaceloma* was found parasitizing the leaves in some of the collections made by me in the Mysore forests. The infected leaves have a warty surface with copper-coloured patches on the upper surface, and tend to roll and curl inwards. In association with *Asterina congesta* Cke. and an *Oidium*, which are also found as leaf parasites on *Santalum album*, the damage caused by the *Sphaceloma* is important, for the affected leaves fall long before healthy leaves. As no detailed search has been made in the sandal-growing areas of Mysore for studying the spread of the disease, it is not possible to evaluate the extent of the damage.

The infection first appears as cinereous areas on the upper surface, which gradually turn purplish to black, appearing as discolorations. The acervuli are intra-epidermal in origin and are distributed on either surface of the eaves. They rarely coalesce, and even where they are in close proximity he demarcation between the individual sori is still visible. The conidiophores are developed from scanty stromata or hyphal cells which become ndistinguishable in later stages. The conidiophores are grouped in plumose ufts (Fig. 1), 43–60μ long and 2–2·5μ broad, hyaline, thin-walled and pointed at the apex. The conidia are produced in succession at the tip. They are hyaline, thin-walled, ovate to ellipsoid (Fig. 2), measuring

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5–9 × 2·5–3 μ. No species of *Sphaceloma* has so far been described on any of the members of the Santalaceae, and comparison with the described species indicates that the species on *Santalum* is new.

Sphaceloma Santali Thirumalachar, n.sp.

Producing cinereous patches on the upper leaf surface, sometimes with purplish to black tinge and appearing as brown to black discolorations. Acervuli amphigenous, but mostly epiphyllous, intra-epidermal, 60–140 μ broad, 43–60 μ high, not coalescing with one another; conidiophores 43–60 μ long, produced in plumose tufts and developed from scanty stromata or hyphal cells which are indistinguishable later on; conidia produced acrogenously, hyaline, ovate-elliptic, measuring 5–9 × 2·5–3 μ.

Habitat. On the leaves of *Santalum album* L., Lakkavalli, Mysore State, leg. M. J. Thirumalachar, 14 October 1945. Type deposited in the Herb. Crypt. Ind. Orient., New Delhi, in the Imperial Mycological Institute, Kew, England, and in the Instituto Biologico, Sao Paulo, Brazil.

Producit cinereas maculas in superiore facie foliorum, nonnumquam pallide purpurescentes vel nigras, quae et brunneo-nigrae discolorationes apparent. Acervuli intra-epidermales, amphigeni sed ut plurimum epiphylli, latitudine 60–140 μ, altitudine 43–60 μ, haud coalescentes inter se; conidiophori 43–60 μ longi, plumose caespitosi, evoluti ex stromatis vel hypharum exiguis cellulis quae postea indistinctae evadunt, 43–60 μ longi, apice acuto; conidia acrogene producti in apice, hyalina, ovata vel elliptica, magnitudinis 5–9 × 2·5–3 μ.

In foliis *Santali albi* L., Lakkavalli, Mysore State, leg. M. J. Thirumalachar, 14. 10. 1945.

(2) A NEW *SPHACELOMA* ON *OSYRIS WIGHTIANA* WALL.

Osyris Wightiana, a member of the Santalaceae, is a partial root parasite in the hilly districts of Mysore. The heart wood of the plant is very faintly fragrant, and is often used for adulterating sandal wood. Some collections of the leaves of this plant made near Nandi Hills, Mysore State, possessed concentric leaf spots in association with the epiphyllous ascomycetous fungus *Meliola Osyridicola* Hansf. which forms large sooty blotches masking the infection spots of the *Sphaceloma*. The infection spots are minute, amphigenous, arranged in perfect concentric rings (Fig. 3). The oldest acervuli are situated in the centre, the infection spreading centrifugally. Macroscopically, the acervuli appear as minute depressions on the surface of the leaves.

Sections through the infection spot show that the sori are intra-epidermal, formed by the grouping of a stroma of hyphal strands (Fig. 4). The cells of the host beneath the sori become depleted of their contents and turn orange coloured and canker-like. Palisade layers of conidiophores that are differentiated from the stroma, break through the upper wall of the epidermis and the cuticle, and abstrict off conidia. The mature conidia are hyaline, one-celled, ovate to globose (Fig. 5) and measure 3 × 2·5 μ. This species of *Sphaceloma* differs from *S. Santali* in the nature of the sori and other characters, such as the length of the conidiophores.

Sphaceloma Osyridis Thirumalachar, n.sp.

Infection spots amphigenous, appearing as minute depressions, arranged in perfect concentric rings. Acervuli intra-epidermal, composed of strands of hyphae and a palisade layer of conidiophores, not coalescing with each other and not becoming continuous. Conidia hyaline, acrogenous, ovate to spherical, thin-walled, measuring 3 × 2·5 μ.

Habitat. On the leaves of *Osyris Wightiana* Wall., Nandi Hills, Mysore State, leg. M. J. Thirumalachar, 19 February 1944. Type deposited in the Herb. Crypt. Ind. Orient., New Delhi, in the Imperial Mycological Institute, Kew, England, and in the Instituto Biologico, São Paulo, Brazil.

Infectionis maculae amphigenae, apparentes ut minutae depressiones, dispositae in circulis perfecte concentricis. Acervulus intra-epidermalis, constans ex hypharum fasciculis atque strato conidiophororum, non coalescens cum aliis nec cum illis continuus evadens. Conidia hyalina, acrogena, ovata vel sphaerica, tenuiter parietata, magnitudinis 3 × 2·5 μ.

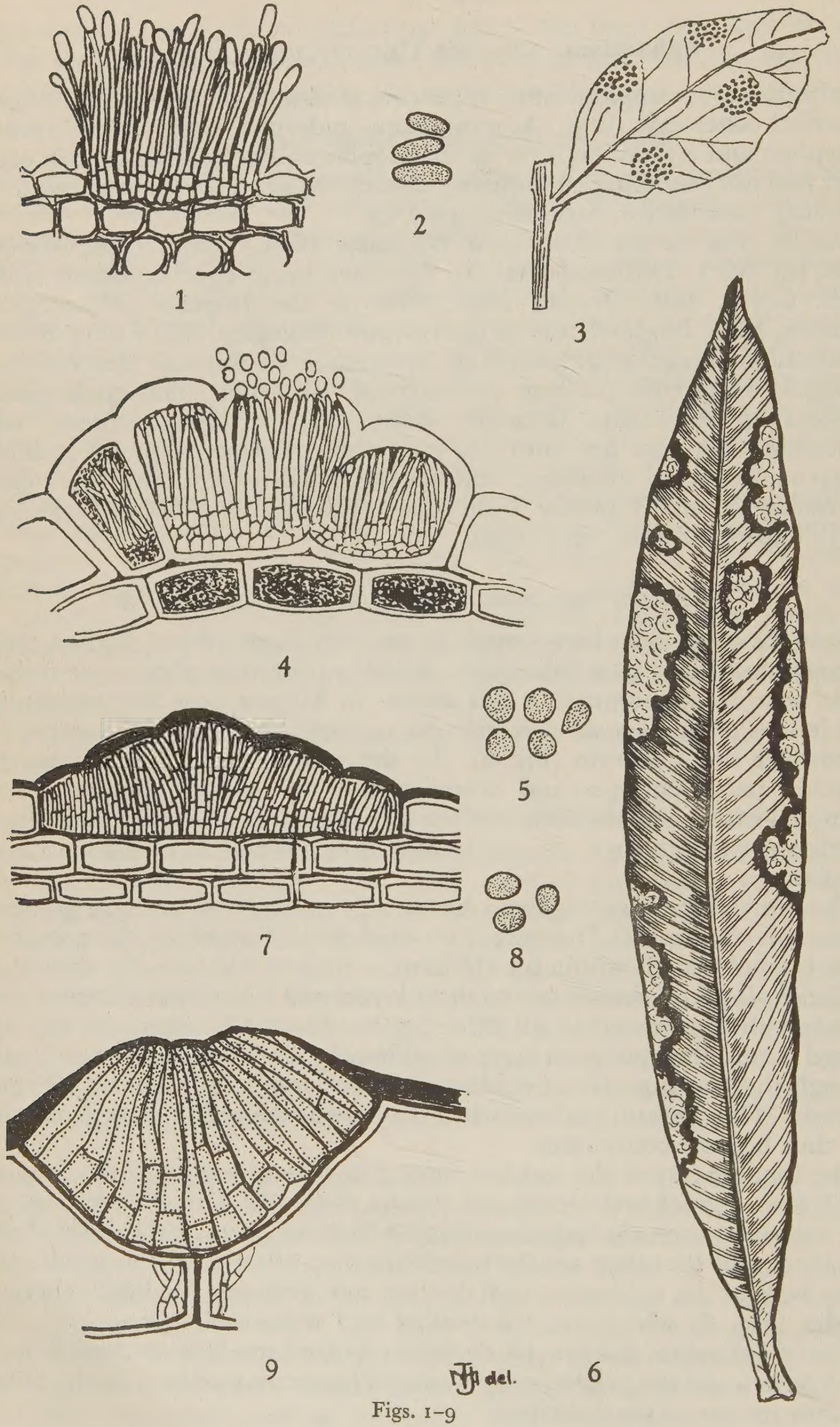
In foliis *Osyridis Wightiana* Wall. in loco Nandi Hills, Mysore State, leg. M. J. Thirumalachar, 19. 2. 1944.

(3) AN ANTHRACNOSE DISEASE OF OLEANDER LEAVES

Collections of oleander leaves made by me near Koppa Road, Mysore, were severely parasitized by a *Sphaceloma*. Another collection of the same fungus made near Kemmangundi, a hill station in Mysore, was also examined. The lesions on the leaves were circular to irregular, whitish, bordered by a brownish black margin (Fig. 6). By the coalescence of these lesions, the infection patch enlarges and occupies most of the leaf surface. When examined with a lens, the surface of the infected patch presents a punctiform to verrucose appearance. In severe infection, there is heavy defoliation of the plants.

The acervuli are amphigenous but mostly epiphyllous and are grouped on the infection spots. They are intra-epidermal, formed by the grouping of the hyphal strands within the epidermis. As is well known, the epidermis in *Nerium oleander* possesses two to three layers and it has been observed that the acervuli are formed in all three layers. Especially when the sori are formed within the innermost layer of epidermis, the developing sorus breaks through the two superposed epidermal layers, appearing as subepidermal in origin. The acervuli coalesce when they are developed in close proximity and thus become continuous.

The fundaments of the sori are formed by the grouping of the hyphae which form a thick well-developed stroma of several tiers of cells (Fig. 7). The conidiophores are indistinguishable from the rest of the cells of the stroma, except that they are the terminal group of cells. These rupture the upper wall of the epidermis and develop acrogenously hyaline, spherical conidia (Fig. 8) which are thin-walled and measure 4–5 × 2·5–4 μ. No species of *Sphaceloma* has previously been reported to occur on *Nerium* or its allied genera and the present species cannot be accommodated in any of the previous species so far described.



Figs. 1–9

Sphaceloma Oleanderi Thirumalachar, n.sp.

Leaf spots spherical to irregular, densely grouped, 1–4 mm. in diam. distributed over the entire surface of the leaves, whitish with a brownish black margin, slightly elevated and appearing rugged owing to the punctiform masses of *Sphaceloma*. Acervuli are intra-epidermal, formed in the first, second or the third layers of the upper epidermis, seldom amphigenous, often becoming coalescent, 140–200μ in diam. and 60–85μ high; stroma well developed with several tiers of cells; conidiophores short, 5–6·5 × 2–2·5μ, breaking through the upper wall, developing acrogenously hyaline, thin-walled conidia which measure 4–5 × 2·5–4μ.

On the leaves of *Nerium Oleander*, Koppa Road, leg. M. J. Thirumalachar, 3 April 1945, leg. H. C. Govindu, Kemmangundi 10 October 1945 (Type). Type deposited in the Herb. Crypt. Ind. Orient., New Delhi, in the Imperial Mycological Institute, Kew, England, and in the Instituto Biologico, São Paulo, Brazil.

Foliorum maculae sphaericae vel irregulares, dense aggregatae, 1–4 mm. diam., dispersae per totam foliorum faciem, albidae margine fusco-nigro, tenuiter elevatae atque asperae ob punctiformes massas Sphacelomatis. Acervuli intra-epidermales, producti intra primum, secundum vel tertium stratum epidermatis superioris, raro amphigeni, saepe inter se confluentes, ut plurimum 60–85 × 140–200μ, stromate bene evoluto multiplice cellularum ordine; conidiophori breves, 5–6·5 × 2–2·5μ. perforantes exteriorem epidermatis parietem; conidia acrogene producta, hyalina, tenuiter-parietata, magnitudinis 4–5 × 2·5–4μ.

In foliis *Nerii Oleanderi*; Koppa Road, Mysore, leg. M. J. Thirumalachar, 3. 4. 1945; leg. H. C. Govindu, Kemmangundi, 10. 10. 1945 (Typus).

(4) A LEAF-SPOT DISEASE OF *CURCUMA* CAUSED BY A SPECIES OF *SPHACELOMA*

Indigenous species of *Curcuma* are well distributed in the forests of Mysore. The rhizomes of many of these wild turmerics are used as an additional source of starch by the local people. In one species of *Curcuma* it was observed that the leaves had developed numerous small lesion spots, which on examination proved to be caused by an undescribed species of *Sphaceloma*. The lesions are brownish yellow, circular to ovate, 2–4 mm. in diam. and mostly found on the upper surface.

Sections through these infection spots revealed that the acervuli are subcuticular (Fig. 9) and very rarely, and then probably exceptional,

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Legends for Figures 1–9

Fig. 1. Acervulus of *Sphaceloma Santali*. (× 500.)
Fig. 2. Spores of *Sphaceloma Santali*. (× 1000.)
Fig. 3. Showing the infection spots of *Sphaceloma Osyridis*. (Nat. size.)
Fig. 4. Acervulus of *Sphaceloma Osyridis*. (× 500.)
Fig. 5. Spores of *Sphaceloma Osyridis*. (× 1500.)
Fig. 6. Anthracnosed leaf of *Nerium Oleander*. (Nat. size.)
Fig. 7. Showing the intra-epidermal acervulus in *Sphaceloma Oleanderi*. (× 250.)
Fig. 8. Spores. (× 1000.)
Fig. 9. Acervulus of *Sphaceloma curcumae*. (× 800.)

intra-epidermal. A compact layer of somewhat pale pseudoparenchymatous cells is produced. From this basal stroma, palisade layers of conidiophores are differentiated which are fusiform and become pointed at the apex. The conidiophores become laterally united and thus present a compact appearance. Most of the acervuli were found to be immature and only very young spores in the process of abstriction at the tip have been observed. Consequently the size and shape of the mature conidia remain unknown. The host cells and the entire stroma of the acervulus contained numerous microconidia. Such microconidia were found by Jenkins and Bitancourt in a number of species of *Elsinoe*. This is apparently the first record of *Sphaceloma* on the Zingiberaceae.

Sphaceloma Curcumae Thirumalachar, n.sp.

Acervuli subcuticular, epiphyllous, round or irregular, minute, 31–50μ in diam., pale yellow, composed of compact pseudoparenchymatous cells with conidiophores which are palisade-like and coalesce laterally, 20–25μ long; conidia not observed, microconidia numerous and filling up the cells.

On leaves of *Curcuma* sp., Kemmangundi, Mysore State, leg. M. J. Thirumalachar, 9 October 1945. Type deposited in the Herb. Crypt. Ind. Orient., New Delhi, Imperial Mycological Institute, Kew, England, and in the Instituto Biologico, São Paulo, Brazil.

Acervuli subcuticulares, epiphylli, globosi vel irregulares, minuti, 31–50μ lati, 31μ alti, pallide lutei, compositi ex compactis pseudoparenchymaticis cellulis, conidiophoris vallatis atque lateraliter coalescentibus, 20–25μ longis; conidia haud visa, microconidia plurima, cellulas implentia.

In foliis *Curcumae* sp., Kemmangundi, Mysore State, leg. M. J. Thirumalachar, 9. 10. 1945.

REFERENCES

Jenkins, A. E. (1936). A *Sphaceloma* on fruit of *Hesperethusa crenulata*, a remote *Citrus* relative from India. *Phytopathology*, xxvi, 71–3.

Jenkins, A. E. & Fawcett, H. S. (1933). Records of citrus scab from herbarium specimens of the genus *Citrus* in England and the United States. *Phytopathology*, xxiii, 475–82.

Thirumalachar, M. J. (1946). An undescribed species of *Elsinoe* from Mysore. *Mycologia*, xxxviii, 220–5.

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SOME FUNGAL DISEASES OF BRYOPHYTES IN MYSORE

By M. J. THIRUMALACHAR
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(With 8 Text-figures)

The fungi associated with or parasitizing the thalli of the liverworts have attracted the attention of numerous investigators. A brief review of the various facts pertaining to this subject is given by Nicolas (1932). The fungi are usually found only in association with the gametophytes and it is uncertain if they are weak parasites or symbionts. The possible occurrence of a mycorrhizal fungus within the thallus of *Marchantia* was pointed out by Kashyap (1916) in India, and recently Mahabale & Bhate (1945) have recorded the presence of a mycorrhizal fungus in the ventral tissue of the liverwort *Fimbriaria angusta* St. As regards the disease-producing fungi on liverworts in India, the life cycle of *Phaeosphaerella Ricciae* F. L. Steph. parasitizing the gametophytes of *Riccia himalayensis* St. was described by Srinivasan (1939). In the course of the collection of material of Hepaticeae by me for class work, the occasional presence of fungi as parasites was noticed. Since no fungi were suspected to be present at the time of collection, the materials were preserved in formalin alcohol and consequently they were unfit for undertaking any germination or cultural studies.

(1) A *CLADOCHYTRIUM* PARASITIC ON *ANEURA* SP.

In some of the collections of gametophytes of *Aneura* sp. made near Agumbe, Mysore State, many of the thalli had developed discoloured patches. In place of the healthy green colour, the affected patches had turned straw yellow. A detailed examination of such thalli indicated that they were parasitized by a species of *Cladochytrium*. No species of *Cladochytrium* has so far been recorded from India, but another genus of the family Cladochytriaceae, *Nowakowskiella ramosa* Butler, was reported on decaying wheat stems by Butler (1907).

The fungus is intramatrical, the 'rhizomycelium' pervading the cells of the host and forming extensive branches. At short intervals the rhizomycelium swells up and forms spindle-shaped swellings or the turbinate organs (Figs. 1, 2) which are very characteristic of the family Cladochytriaceae. These spindle-organs are mostly continuous (Fig. 2) without any wall formation, but sometimes (Fig. 1) they are definitely septate. It might therefore be well to consider the spindle organs in the present species as being sparsely septate. In distinguishing between the genera *Cladochytrium* and *Nowakowskiella*, the presence of an operculum in the zoosporangium of the latter is stated to be an important character, and Whiffen (1943) considers that the presence of the septations in the spindle-organs of *Cladochytrium* adds another differentiating character. The spindle-organs of *Nowakowskiella* are therefore regarded as unseptate and continuous.

Zoosporangia and resting spores were observed in large numbers. The zoosporangia are terminal or intercalary, their size and shape varying according to the host cell in which they are lodged. The division of the contents of the zoosporangium into zoospores was frequently noticed (Fig. 3), but their actual discharge could not be followed on account of the preserved state of the material. On some old zoosporangia, small papillate structures which might represent the remnants of the exit tubes were seen. The resting spores are ovate to spherical, cinnamon-yellow, thick-walled and smooth. The mature spores are often formed by the transformation of the spindle-organs and measure 30–44μ in diam.

While species of *Cladochytrium*, such as *C. replicatum* Karling, are considered to be very weak parasites or almost saprophytes, other species, such as *C. Nowakowskii* Sparrow (Sparrow, 1931), is stated to be a virulent parasite on algae. The discoloration produced in the thalli of *Aneura* by the species under study shows that it might also be a weak parasite. Inoculation experiments carried out by Karling (1931) indicated that the species of *Cladochytrium* possess wide host ranges and the identity of the species therefore on the basis of host alone would be erroneous. *C. replicatum*, for instance, has been successfully inoculated into several hosts, including the liverwort *Anthoceros* (Karling, 1931). That being so, the identity of the species should be based on morphological characters and spore measurements alone. The sparsely septate nature of the spindle-organs, the inconspicuous, papillate exit-tube and the difference in size of the resting spores as compared with those of most of the species of *Cladochytrium*, has led me to the view that the species may be an undescribed one, though future investigations may reveal it to be identical with some well-established species.

Cladochytrium Aneurae Thirumalachar n.sp.

Thallus intramatrical consisting of fine tenuous branched rhizomycelium with numerous spindle-shaped swellings which rarely become septate; zoosporangia spherical to oval, depending upon the shape of the host cell, often with a single constricted exit pore. Resting spores terminal or intercalary, often formed by the transformation of spindle-organs, usually intramatrical, ovoid to spherical, cinnamon-yellow, thick-walled, smooth, measuring 30–44μ in diam.

Habitat. As a weak parasite within the thallus of *Aneura* sp. (Jungermanniales), Agumbe, leg. M. J. Thirumalachar, 4 April 1945. Type deposited in the Herb. Crypt. Ind. Orient., New Delhi, and in the Imperial Mycological Institute, Kew, England.

Thallus intramatricalis constans ex gracili tenuique atque ramoso rhizomycelio ornato plurimis fusiformibus tumoribus qui raro septati evadunt; zoosporangia sphaerica vel ovata pro forma cellulae parasitatae, 52–100μ diam., unico constricto exitus poro. Quiescentes sporae terminales vel intercalares, saepe evolutae ex transformatione fusiformium organorum, plerumque intramatricales, ovatae vel sphaericae, cinnamomo-luteae, crasso pariete ornatae, leves, magnitudis 30–44μ.

Parasitus debilis intra thallum *Aneurae* sp. (Jungermanniales) in loco Agumbe; leg. M. J. Thirumalachar, 4. 4. 1945.

(2) *OLPIDIOPSIS RICCIAE* DU PLESSIS

Olpidiopsis Ricciae Du Plessis was first described by Du Plessis (1933) within the rhizoids of *Riccia* in South Africa. Large numbers of sporangia and oospores were observed by him within the normal and pegged rhizoids. The presence of a companion-cell which in fact represents the empty antheridium attached to the oospore is a characteristic feature. The oospores have been observed within the rhizoids and rarely within the epidermal cells of *Riccia*, and the host range of the fungus is as yet unknown.

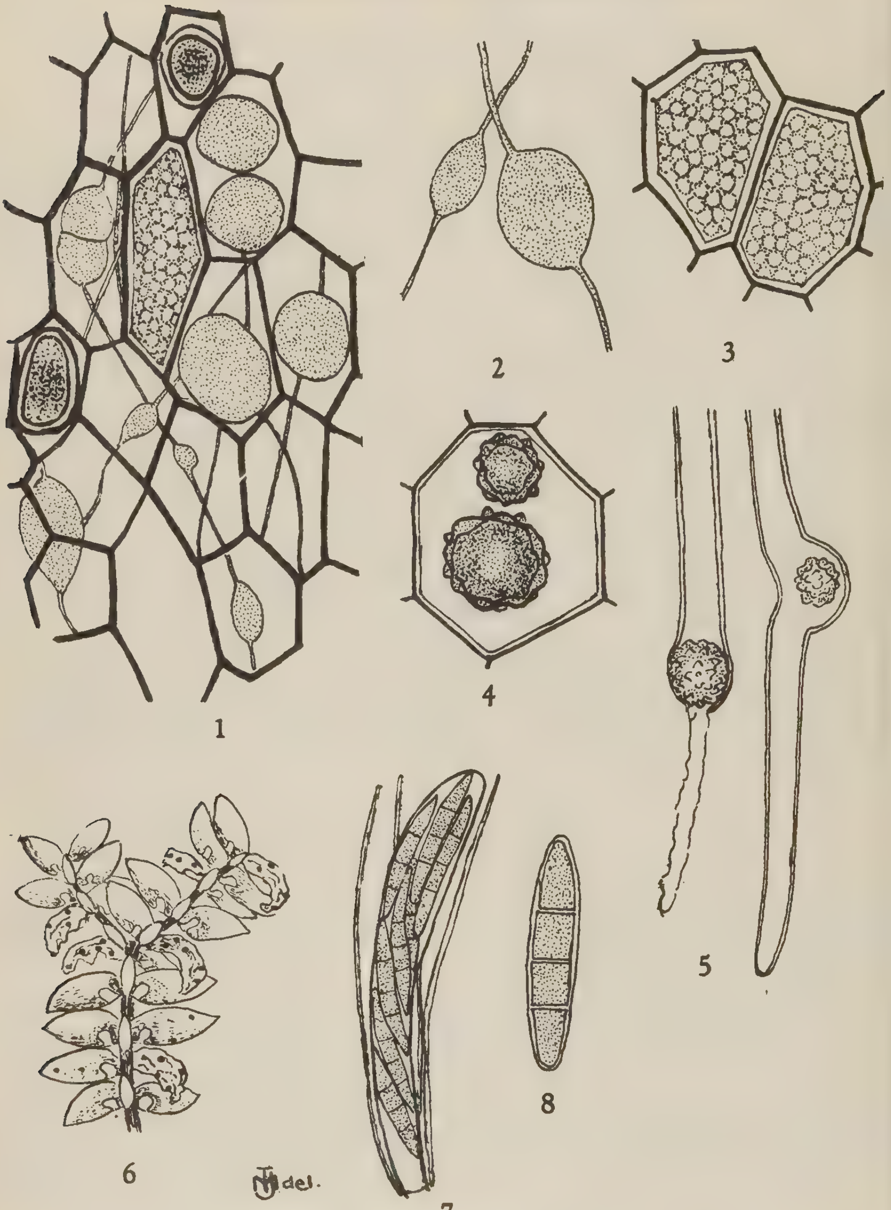
An *Olpidiopsis* which seemed to be identical with *O. Ricciae* Du Plessis was found by me within the rhizoids of many Hepaticeae in addition to *Riccia*. The oospores have been found in the rhizoids of species of *Marchantia*, *Plagiochasma*, *Fimbriaria*, *Aneura* and *Notothylas*. It seems therefore probable that the fungus may have a wide host range.

Most of the oospores found were mature. They are yellowish brown with a sinuous outer wall. The companion-cell is mostly lacking from the mature spores; Du Plessis also records this. Whiffen (1942) states that *Olpidiopsis Aphanomycis* Cornu lacks the characteristic companion-cell. When the oospores are present in large numbers it seems that the cell contents of the rhizoids become depleted and shrivel up. Sometimes, as in the rhizoids of *Aneura*, at the place where the young oospore is lodged, the rhizoid turns and twists, forming a nodular structure indicating a definite necrotic effect (Fig. 5). In other specimens the definite shrivelling up of the rhizoid from the free end is noticeable. It might be of interest to take into account the view put forward by Du Plessis about the relationship. Because there were no visible signs of necrotic effect produced by the oospores, Du Plessis suggested that the relationship between the host and *Olpidiopsis Ricciae* might be one of symbiosis. My observations on what seems to be the same species of *Olpidiopsis* indicate that there is weak parasitism as in *Cladochytrium*, becoming discernible in certain hosts or when the host is not able to offer resistance.

In studying the host ranges for the species of *Olpidiopsis*, both Shanor (1940) and Whiffen (1942) pointed out that it is impossible to transfer any species of *Olpidiopsis* from the species of host on which it was collected to the host of any other species. No such studies have been made in the present investigation to confirm this interesting observation, but, because of the close morphological resemblance of the oospores found in the rhizoids of the various liverworts, the fungus in all the hosts is referred to *O. Ricciae*.

(3) A *LEPTOSPHAERIA* PARASITIC ON *PORELLA*

Species of *Porella* are very commonly found as epiphytes on the branches and leaves of forest trees. In some of the collections of *Porella* made in Nandi Hills, Mysore State, the leafy lobes of the gametophyte were noticed to have been parasitized by an ascomycetous parasite, the infected leaves wilting away and finally drying up. Examination of the infected



Figures 1–8

Fig. 1. Showing intramatrical thallus of *Cladochytrium Aneurae*. (× 400.)
Fig. 2. Some of the unseptate spindle-organs. (× 600.)
Fig. 3. Stages in the formation of zoosporangium. (× 800.)
Fig. 4. Oospores of *Olpidiopsis Ricciae* within ventral tissue *Aneura*. (× 1000.)
Fig. 5. Oospores of *Olpidiopsis Ricciae* within the rhizoids. (× 500.)
Fig. 6. Gametophyte of *Porella* with *Leptosphaeria* infection spots. (× 3.)
Fig. 7. Ascus of *Leptosphaeria Porellae*. (× 800.)
Fig. 8. Ascospore. (× 1800.)

leaves with a field lens, reveals numerous black specks distributed on the dorsal and ventral lobes as well as the amphigastria of the thallus (Fig. 6). The infection has been traced to a species of *Leptosphaeria*. The mycelium is intercellular, slightly dark, closely septate and ramifies within the tissues of the mesophyll. Numerous perithecia are distributed on the surface of the infected portions. They are minute, black, immersed within the thallus with the ostioles projecting as minute black specks. The asci are cylindrical, obovate at the apex, and associated with paraphyses. The ascospores are fusiform, rounded at both ends, hyaline, and usually three-septate. The spores are arranged in a biseriate manner and measure 15–20 × 2·5–3·5 μ (Figs. 7, 8). The association of the *Phoma* stage of the fungus with hyaline spores was observed. As no species of *Leptosphaeria* is so far known on any of the species of *Porella* or any other liverworts, it is not possible to institute any comparison. The name *Leptosphaeria Porellae* is proposed for the fungus.

Leptosphaeria Porellae Thirumalachar, n.sp.

Perithecia on the foliose portions of the thallus, amphigenous, immersed in the early stages, later on becoming erumpent; black, spherical, ostiolate, ostiole projecting as a small papilla. Asci cylindric, 8-spored, 57–66 × 6·5–7·5 μ, paraphysate; paraphyses numerous, simple, filiform; spores one to two-seriate, pale yellow, fusoid, rounded at both ends, smooth, slightly or not constricted at the septa, measuring 15–20 × 2·5–3·5 μ.

Habitat. On the thallus and causing the wilting of *Porella* sp., Nandi Hills, Mysore State, leg. M. J. Thirumalachar, 10 February 1944. Type deposited in the Herb. Crypt. Ind. Orient., New Delhi, and in the Imperial Mycological Institute, Kew, England.

Perithecia super foliosas partes thalli, amphigena, initio immersa, postea erumpentia, nigra, sphaerica, ostiolata, ostiolo eminente ut papilla parva. Asci cylindrici, 8-spori, 57–66 × 6·5–7·5 μ, paraphysati; paraphyses plures, simplices, filiformes; sporae semel vel bis seriatae, pallide luteae, fusiformes, utroque apice rotundatae, leves, tenuiter vel nullo modo constrictae ad septa, magnitudinis 15–20 × 2·5–3·5 μ.

In thallo *Porellae* spec., quem marcescentem reddit; in loco Nandi Hills, Mysore State, leg. M. J. Thirumalachar, 10. 2. 1944.

In conclusion the writer wishes to express his deep debt of gratitude to Rev. Father H. Santapau, Professor of Botany, St Xavier's College, Bombay, for kindly rendering into Latin the diagnosis of the new species. Grateful thanks are due to Dr B. B. Mundkur and Dr L. N. Rao for kind encouragement and valuable suggestions.

REFERENCES

BUTLER, E. J. (1907). An account of the genus *Pythium* and some Chytridiaceae. *Mem. Dep. Agric. India*, I, 137–41.

KARLING, J. S. (1931). Studies in the Chytridiales. VI. The occurrence and life history of a new species of *Cladochytrium* in cells of *Eriocaulon septangulare*. *Amer. J. Bot.* XVIII, 526–57.

KASHYAP, S. (1916). *J. Bombay Nat. Hist. Soc.* XXIV, 349.

MAHABALE, T. S. & BHATE, P. D. (1945). The structure and life history of *Fimbriaria angusta* St. *J. Bombay Univ.* XIII, 5–15.

NICOLAS, G. (1932). Association des Bryophytes avec d'autres Organismes. In *Manual of Bryology*, edited by Fr. Verdoorn, Hague, pp. 109–28.

DU PLESSIS, S. J. (1933). The life history and morphology of *Olpidiopsis Ricciae* nov.sp. infecting *Riccia* sp. in S. Africa. *Ann. Bot., Lond.*, XLVII, 755–62.

SHANOR, LELAND (1940). Studies in the genus *Olpidiopsis*. III. Some observations on the host range of certain species. *J. Elisha Mitchell Sci. Soc.* LVI, 165–76.

SPARROW, F. K. (1931). Two new Chytridiaceous fungi from Cold Spring Harbor. *Amer. J. Bot.*, XVIII, 615–22.

SRINIVASAN, K. S. (1939). An ascomycetous fungus attacking *Riccia Himalayensis* St. *Trans. Brit. Myc. Soc.* XXIII, 55–62.

WHIFFEN, ALMA J. A. (1942). A discussion of some species of *Olpidiopsis* and *Pseudo-olpidium*. *Amer. J. Bot.* XXIX, 607–11.

WHIFFEN, ALMA J. A. (1943). New species of *Nowakoskiella* and *Blastocladia*. *J. Elisha Mitchell Sci. Soc.* LIX, 37–43.

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THE AMBROSIA FUNGUS OF *XYLEBORUS FORNICATUS* EICH.

By C. H. GADD and C. A. LOOS, *Tea Research Institute of Ceylon*

(With Plate I and 26 Text-figures)

Many beetles of the family Scolytidae have long been known to live in association with specific fungi, the so-called 'ambrosia', which form the main, and possibly the sole food supply of their larvae. Yet little is known of these fungi, as may be seen from the review of the literature given by Leach, Hodson, Chilton & Christensen (1940).

Xyleborus fornicatus Eich., the shot-hole borer of tea, is an important ambrosia beetle pest in many tea districts of Ceylon, because the female beetles make galleries in living tea stems, which in consequence tend to break rather easily and so cause loss of crop. The beetle also invades other plants, of which the most important is the castor-oil tree. The beetle from tea is smaller than that from castor, measuring about 2·3 mm. in length as compared with 2·5 mm. from castor, and its elytral curve is more convex from scutellum to sutural apex. Because of these morphological differences, which, however, are not constant, Eggers (1922) described specimens from tea as a new species, *X. fornicatior*. Sampson (1923) and Beeson (1925) were unable to accept *X. fornicatior* as a valid species, though Beeson considered the retention of the name in subspecific rank to be desirable on biological evidence. Beeson (1941) stated that in north-east and north-west India and in Indo-China the tea bush is rarely attacked by *X. fornicatus*, although the borer is abundant in other hosts in the same locality, and there is no trace of the *fornicatior* characters. In Ceylon (Gadd, 1946) experiments have demonstrated that the beetle from castor will bore into tea stems and the beetle from tea into castor stems, but whether the host plants are normally interchangeable in nature seems doubtful. Nor, by any means, do the beetles from tea always show *fornicatior* characters; they are more often indistinguishable from castor beetles. For this reason the specific name *fornicatus* will be used here throughout to avoid any implication that galleries in tea stems had been made by beetles with *fornicatior* characters.

The shape and size of galleries vary to some extent with the size of the branch in which they are made. Tea stems about half an inch in diameter and less seem to be preferred, whereas castor stems are usually much thicker when attacked. Galleries are made by females alone without any assistance from males. Each starts with a straight entrance gallery of variable length running radially into the stem. In small tea stems the entrance gallery stops at the pith, along which a straight longitudinal gallery is then made. More frequently, especially in thicker stems, the entrance gallery terminates in the wood near the cambium and the branch galleries are circular, running parallel with the outer surface of the stem, often very close to the cambium yet not damaging it. All galleries are communal, there being no special brood cells or chambers. Eggs are usually found near the gallery ends, often in small heaps of five or six, but larvae, pupae and adults may be located in any part of a gallery.

Ambrosia can be seen as a fine, white, frost-like dust on the gallery wall, particularly where young larvae are feeding. It is not usually visible on the dark-stained walls of old galleries; nor does it ever occur in such quantity as to block the passage. There is no carefully prepared bed or layer of chips and excreta for the fungus to develop on, as mentioned by Imms (1934, p. 531) in his short general account of ambrosia, nor can two superimposed fungal layers be distinguished as described by Trotter (1934) from galleries made by an unnamed beetle in branches of living *Brownea grandiceps*.

Sections show that the fungus consists of a thin surface layer from which arise numerous short, erect, unbranched, septate conidiophores, at the ends of which are solitary club-shaped spores, aseptate at first but with several (up to five) transverse septa later (Pl. I, fig. 1). This fungus belongs to the genus *Monacrosporium*. Amongst the conidiophores, close to the gallery walls, a few globose cells about 30 μ in diameter have been observed in tea stems, but not in castor. When stained, these globose cells each contain what appears to be a two-celled body (Text-figs. 1, 2). The nature of these cells is not understood and they do not occur in pure cultures of the *Monacrosporium*. In galleries in castor the conidiophores are somewhat more septate and the spores shorter and broader, but in other respects the fungi appear alike. Spores from galleries in tea (Text-figs. 3–5) range from 34–51 × 10·5–13 μ, while those from castor (Text-figs. 11, 12) range from 25–37 × 11–15·5 μ. Mean measurements of fifty spores were 40 × 11·5 μ from tea, and 32 × 13 μ from castor.

Speyer (1918), in a short statement on the fungi from shot-hole borer galleries in tea stems, wrote: 'Observations on the fungus upon which the larvae of shot-hole borers feed show that two fungi are generally present in healthy galleries. These have been identified by Mr Petch as *Monacrosporium* and a conidial ambrosia fungus. The spores of the former appear in the gallery about seven days after the beetle has entered the branch. It is known that the conidial stage of some ambrosia fungi are modified stages of fungi which grow free, on certain media, in a totally different form; there is therefore a possibility, though a remote one, that *Monacrosporium* is the free-living phase of the ambrosia grown by shot-hole borer.' In a later publication (1923) he gave another description: 'The ambrosia mycelium grown by *X. fornicatus* consists of comparatively long, sparsely septate, narrow hyphae; curiously enough, the rounded conidial bodies characteristic of other ambrosia fungi have not been observed. In addition, spores of *Monacrosporium* are constantly found in the tunnels when this insect is excavating the gallery, and this fungus occurs also amongst the ambrosia hyphae.'

Speyer was evidently of the opinion that two fungi occur in the galleries, though how they can be distinguished in the absence of 'rounded conidial bodies characteristic of other ambrosia fungi' is not apparent. Whether the globose cells amongst the conidiophores previously referred to in galleries in tea belong to a distinct fungus has not been determined, and they have not been observed in cultures of *Monacrosporium*. The available evidence indicates, however, that they are of no vital importance in insect nutrition.

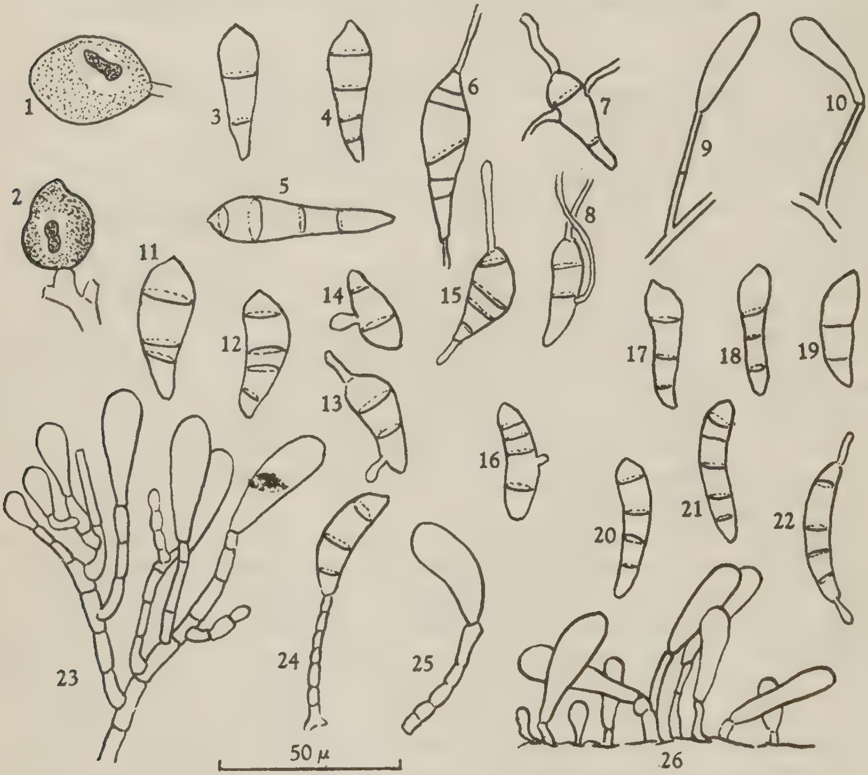
If by the term ambrosia is meant the actual food of beetle larvae, then the *Monacrosporium* spores are ambrosia. The spores can be found in considerable numbers in the anterior gut of larvae freshly removed from galleries, and newly hatched larvae have been brought through all stages to pupation on pure *Monacrosporium* cultures derived from castor galleries in tea and castor. Nothing other than the fungus appears essential for their growth.

Single-spore cultures were easily obtained by plating spores from freshly opened galleries in tea or castor stems. The spores germinate rapidly, aseptate spores becoming septate and forming germ tubes within four hours. Germ tubes are normally produced at the apical and basal cells though they may arise from any cell (Text-figs. 6–8, 13–16). The fungus grew well on both malt agar and Waksman's agar without acid (Waksman, 1927, p. 19) at room temperature at the St Coombs Laboratory (64–76° F.). These were the only culture media tested, but it is probable that others would be equally suitable.

The cultures obtained from spores in galleries in tea were easily distinguished from those obtained from castor by their slower growth and greater colour development. At 26° C. cultures from tea, on plates, grew at an average rate of rather more than 5 mm. per day, whereas the castor cultures grew almost 9 mm. per day. Castor cultures were distinctly zoned (Pl. I, fig. 3) and had regular smooth edges, whereas tea cultures were less distinctly zoned with broken, rugged edges, and were markedly coloured (Pl. I, fig. 2). In tubes, the aerial mycelium of castor cultures was more cotton-woolly than those from tea, which had a looser and shorter mycelium. The colour change in plate cultures from tea became noticeable about the fourth day when the aerial mycelium near the centre of the colony became flesh coloured. Later the colour deepened to salmon, but the growing edge remained white. This colour development was not noticeable in tube cultures, nor in any cultures from castor. The agar of tea cultures, particularly slants, becomes deeply stained, dark vinaceous, near Corinthian purple (Ridgway, pl. 38), whereas in castor cultures the colour never attained a similar depth; it approximated Daphne red (Ridgway, pl. 38).

It may be appropriate to mention here that the wood adjacent to galleries in tea stems is usually stained dark purple (Speyer, 1922), though some variation occurs both in the colour and amount of it. The wood around galleries in castor is, however, rarely coloured. These observations support Verrall's (1943) statement that the stain around beetle tunnels is caused by the ambrosia fungi, which may easily be isolated from such wood. He attributed the intensification of colour to reactions of these fungi with insect secretions and other organisms, but in the writers' opinion it may be due largely to the strain of the ambrosia carried by the beetles.

In microscopic detail very little difference was to be observed between the two cultures. The spores are normally produced on short unbranched conidiophores, though in tubes in which larvae had fed branched conidiophores were encountered, each branch terminating with a solitary conidium (Text-fig. 23). The conidiophores are very variable in length, usually much longer and less septate than those observed in galleries. Spores varied somewhat in shape, but were generally clavate with 2–5 septa.



Text-figs. 1–26. *Monacrosporium ambrosium* n.sp.

Text-figs. 1, 2. Globose bodies from galleries in tea stem.
Text-figs. 3–5. Mature spores from tea.
Text-figs. 6–8. Germinating spores from tea.
Text-figs. 9, 10. Immature spores and conidiophores from culture (tea).
Text-figs. 11, 12. Mature spores from castor.
Text-figs. 13–16. Germinating spores from castor.
Text-figs. 17–19. Mature spores from culture (tea).
Text-figs. 20, 21. Mature spores from culture (castor).
Text-fig. 22. Germinating spore from castor culture.
Text-fig. 23. Fragment from culture on which larvae had fed, showing branched conidiophores and immature spores.
Text-figs. 24, 25. Spores and conidiophores from castor gallery.
Text-fig. 26. Immature spores in tea gallery.

The spores from tea were not noticeably larger than those from castor, though fifty measurements of each gave the following results: tea 28–43 × 8–12 μ (mean 36 × 9·5 μ); castor 22–40 × 7–10·5 μ (mean 33 × 8·5 μ).

Spore production was markedly stimulated by removing aerial mycelium with a sterile bent needle. After twenty-four hours a large number of small pustules of spores appeared on the agar surface. Aerial mycelium did not again grow from the cleared surface, but the pustules or globular masses of spores increased in size (Pl. I, fig. 4). Cultures from tea and from castor behaved alike in this respect. The same thing probably occurs in the galleries. The larvae feed mainly, if not solely, on spores, as spores alone have been found in the gut of larvae examined. Their feeding evidently stimulates spore production, for it is in those parts of a gallery where larvae are feeding that spores can be found in large numbers.

In feeding experiments larvae hatched from eggs on damp blotting paper have been transferred to tea and castor cultures. Larvae from both sources fed readily on both cultures, and it appeared immaterial whether the tea or castor ambrosia was used as food supply for either beetle. Larvae were successfully brought to the pupal stage on both cultures.

Experiments clearly demonstrated that the food of *Xyleborus fornicatus* is the fungus *Monacrosporium* described. How the parent beetle carries it from the original gallery and plants it in her new gallery is not known. Several hypotheses have been advanced, but they need not be considered here. The importance of the fungus in the insect's economy, and the amount and nature of the work to be done before it can be planted in a suitable place for growth, seem to rule out the possibility that transfer is entirely accidental. But whether the transfer is accidental or due to instinctive actions, there can be little opportunity for the intermixing of the fungi carried by different families. It would not be surprising therefore, if numerous strains of the fungus exist, differing from one another in minor characters, such as colour development, determinable in comparable artificial culture. For this reason we regard the differences observed in culture between the two strains used in these studies as of minor importance.

So far as we are aware, this fungus has not been described previously; nor do we know of another ambrosia fungus belonging to the genus *Monacrosporium*. We therefore propose for it the name *Monacrosporium ambrosium*.

Monacrosporium ambrosium n.sp.

The ambrosia fungus of the beetle *Xyleborus fornicatus* Eich. Vegetative hyphae in the wood adjacent to the galleries and thinly lining the gallery walls, thin, hyaline, septate and branched. Conidiophores produced on the gallery walls, crowded, erect, septate, normally unbranched, rarely exceeding the spore in length. Spores solitary, terminal, hyaline, clavate, non-septate at first, with rounded apex, later and before germination becoming 2–5 septate and with a bluntly pointed apex, 25–51 × 10·5–15·5 μ.

Habitat. In galleries of *Xyleborus fornicatus* Eich. in *Camellia sinensis* (tea), *Ricinus communis* (castor-oil tree) and other stems invaded by the beetle.

Hyphae steriles in ligno contiguo, etiam in parietibus internis cuniculorum effusae, tenues, hyalinae, septatae, ramosae. Conidiophora in cuniculis efformata, dense congregata, erecta, septata, plerumque haud ramosa, longitudine rare sporam excedentia, apice monospora. Conidia hyalina, clavata, primo continua apice rotundata, demum ante germinationem 2–5 septata, in apicem obtusum angustata, 25–51 × 10·5 – 15·5μ.

Hab. in cuniculis *Xylebori fornicati* Eich., in caulibus *Camelliae sinensis*, *Ricini communis* et arborum aliarum.

We are indebted to Miss E. M. Wakefield, M.A., F.L.S., of the Herbarium, Royal Botanic Gardens, Kew, for the Latin diagnosis.

SUMMARY

The fungus commonly associated with the ambrosia beetle *Xyleborus fornicatus* Eich., the shot-hole borer of tea and other plants, is described as new, and named *Monacrosporium ambrosium*. This fungus is undoubtedly the main, if not the sole, food of the beetle, as larvae have been brought from the egg to pupation on cultures of the fungus.

REFERENCES

Beeson, C. F. C. (1925). *Xyleborus fornicatus* in India. *Trop. Agriculturist*, lxv, 371–2.

Beeson, C. F. C. (1941). *The Ecology and Control of the Forest Insects of India and the Neighbouring Countries*. Dehra Dun.

Eggers, I. H. (1922). Kulturschädliche Borkenkäfer des indischen Archipels. *Ent. Ber. Amst.* vi, no. 126, pp. 84–8. Abstract in *Rev. appl. Entom.* A, x, 572.

Gadd, C. H. (1946). Studies of shot-hole borer of tea. 1. Distribution and nomenclature. *Tea Quart.* xviii, 46–54.

Imms, A. D. (1934). *A General Text Book of Entomology*, 3rd ed. London.

Leach, J. G., Hodson, A. C., Chilton, St J. P. & Christensen, M. (1940). Observations on two ambrosia beetles and their associated fungi. *Phytopath.* xxx, 227–36.

Sampson, F. W. (1923). Notes on the nomenclature of the family Scolytidae. *Ann. Mag. nat. Hist.* xi, no. 62, pp. 269–71. Abstract in *Rev. appl. Entom.* A, xi, 257.

Speyer, E. R. (1918). Progress report on investigations into shot-hole borer of tea. *Trop. Agriculturist*, l, 373–4.

Speyer, E. R. (1922). Shot-hole borer of tea: Damage caused to the tea bush. *Bull. Dep. Agric. Ceylon*, no. 60.

Speyer, E. R. (1923). Notes upon the habits of Ceylonese Ambrosia beetles. *Bull. ent. Res.* xiv, 11–23.

Trotter, A. (1934). Il fungo-ambrosia delle gallerie di un Xyleborino di Ceylon. *Ann. Ist. sup. agr. Portici*, 3 Ser. vi, 256–75. Abstract in *Rev. App. Myc.* xiv, 167.

Verrall, A. F. (1943). Fungi associated with certain ambrosia beetles. *J. agric. Res.* lxvi, 135.

Waksman, S. A. (1927). *Principles of Soil Microbiology*. London.

EXPLANATION OF PLATE I

Fig. 1. Section through gallery in tea stem (pith) showing spores. ×280.
Fig. 2. Culture on Waksman's agar 8 days old, tea strain. ×⅓.
Fig. 3. Culture on Waksman's agar 8 days old, castor strain. ×⅓.
Fig. 4. Spore pustules produced after removing aerial mycelium from culture. ×30.

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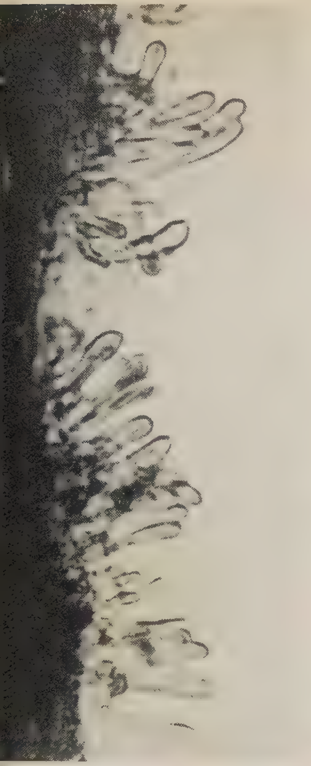

Fig. 1

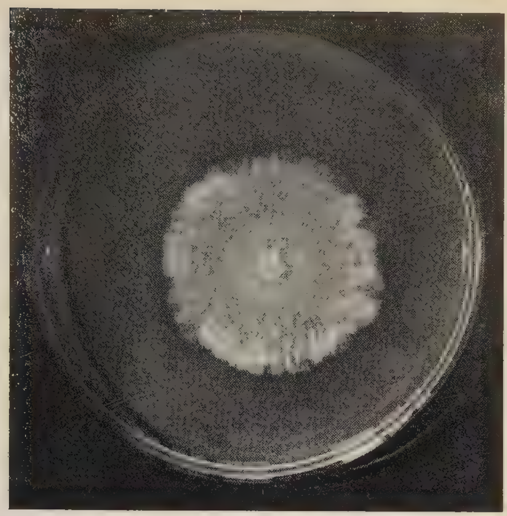

Fig. 2

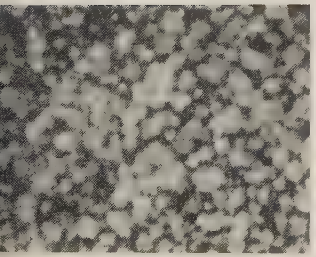

Fig. 4

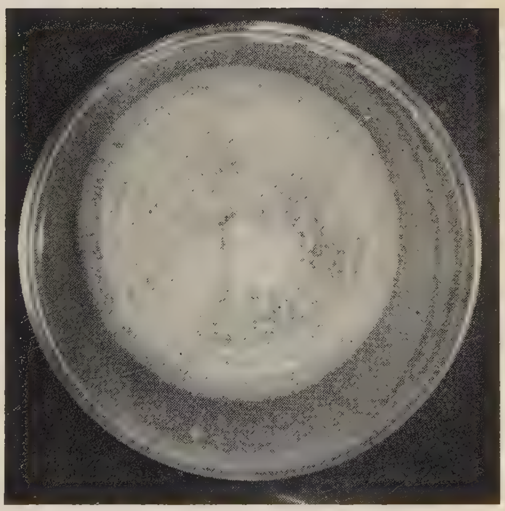

Fig. 3

A REVISION OF CEYLON *MARASMII*

By T. PETCH

(With Plates II–IV)

In their general account of the fungi of Ceylon (*J. Linn. Soc.* xiv, 31), Berkeley and Broome wrote '*Marasmii* and *Lentini* are, as might be expected, abundant', and in their systematic list they enumerated forty-four species of *Marasmius*, thirty-five of them new species. Unfortunately, only thirteen of these were illustrated in the series of paintings of agarics, sent with specimens by Thwaites and now at Peradeniya, from which Berkeley and Broome described most of their Ceylon species. There are also at Peradeniya specimens of Ceylon fungi which were erroneously supposed to have been named by Berkeley and Broome, cotypes in fact, but a later examination of some Thwaites correspondence showed that they had not been seen by them. As with other cryptogams, Thwaites divided each of his gatherings into two parts, one of which was sent to Berkeley and Broome, while the other was retained at Peradeniya, mounted on paper duly numbered, the names being added later after the publication of the 'Fungi of Ceylon'.

Thwaites collected his specimens over a fairly long period, and he placed under the same number those he thought were the same species. Naturally in some instances he was mistaken, and Berkeley and Broome divided up his specimens if they thought that was so. For example, Thwaites's no. 101 was collected on four dates ranging from November 1867 to September 1868, and Berkeley and Broome divided it into four *Marasmii* and a *Collybia*. But there was only one figure, and one can only surmise that the other species resembled it sufficiently closely to make Thwaites think that they were the same. It proved impossible to sort out these and similar mixtures from the specimens at Peradeniya, and the revision of the Ceylon *Marasmii* had to be postponed until Berkeley's specimens in Herb. Kew. and Broome's specimens in Herb. Mus. Brit. (not always the same) could be examined. Specimens were collected, described, and tentatively named, and during my home leave in 1911 and again in 1915, some progress was made in their determination, but with the expansion of the work of the mycological division this project ultimately lapsed.

The following include redescriptions of most of Berkeley and Broome's Ceylon species. The regions covered were practically those in which Thwaites collected his mycological specimens, but even these have not been exhausted. The low country, in both the wet and the dry zones, has yet to be investigated.

Marasmius nephelodes (B. & Br.) Petch, *Ann. Perad.* iv, 403; *Agaricus* (*Collybia*) *nephelodes* B. & Br., Fungi of Ceylon, no. 105; *Marasmius ochraceus* B. & Br., Fungi of Ceylon, no. 355.

Pileus broadly convex, centre even or irregularly depressed, reddish brown in the centre, ochraceous elsewhere, sometimes feebly zoned, margin sometimes whitish, fleshy, smooth, slightly hoary when young, shining, 2–4 cm. diameter, flesh white, becoming reddish when cut; stalk 4–5·5 cm. long, 3–4 mm. diameter, nearly equal, white, sometimes longitudinally streaked ochraceous, becoming reddish or ochraceous when handled, pruinose or minutely tomentose, tough, hollow, base tomentose; gills pallid, distant, free or slightly adnexed, abruptly truncate behind; spores white, narrow oval, 9–13 × 4 μ. On the ground among grass, solitary or in groups. Frequent on the lawns at Peradeniya (Pl. II, fig. 1).

Gardner's painting no. 70, referred by Berkeley to *Hygrophorus obrusseus* Fr., resembles this species, but as it was said to grow on the ground in woods it may be something else.

Marasmius calvus B. & Br., Fungi of Ceylon, no. 354.

Pileus up to 4·5 cm. diameter, hemispherical then broadly convex, sometimes slightly umbonate, fawn-coloured to ashy, bay brown in the centre, minutely pruinose, irregularly radially striate, becoming sodden and dark brown at the margin and along the striae, flesh white, thin; stalk up to 8 cm. long, 4 mm. diameter, white, longitudinally striate, often twisted, glabrous, white tomentose at the base, brittle, stuffed then hollow, brown internally; gills pallid, broad, distant, rounded behind, adnexed. On the ground among dead leaves. No. 4115, Hakgala, 28 September 1914 (Pl. I, fig. 8).

Berkeley and Broome described this species as 'umber, elegantly varied with radiating lines', and the gills as 'sometimes ventricose, as in the more beautifully painted specimens'. Thwaites's no. 766, however, was a mixture, both as regards specimens and paintings, and the figure elegantly varied with radiating lines is of a young specimen of *M. rivulosus*.

Marasmius purpureo-griseus Petch, *n.sp.*

Fasciculate. Pileus broadly convex, purple grey, paler towards the margin, not sulcate, minutely radially silky, about 1·5 cm. diameter, margin incurved, flesh white, thin; stalk up to 4 cm. long, 1·5 mm. diameter, brownish white, shining, cartilaginous, stuffed then hollow, fistulose; gills crowded, white or cream-coloured, broad, adnate or sinuato-adnate, edge straight. On dead wood, attached by white mycelium. No. 5736, Henaratgoda, 25 June 1918. The specimen became rather dry, before the painting could be made (Pl. III, fig. 7).

Marasmius multijugus (B. & Br.) Petch, in *Trans. Brit. mycol. Soc.* XXVII, 138; *A.* (*Collybia*) *multijugus* B. & Br., Fungi of Ceylon, no. 100.

Usually densely caespitose, sometimes connate at the base or up to half the height; pileus conico-campanulate, apex rounded, margin recurved when old, or convex or almost plane, centre sometimes depressed or almost umbilicate, up to 5 cm. diameter, ashy to red brown, glabrous when moist, innately silky when dry, irregularly plicate half-way to the centre, flesh very thin; stalk up to 10 cm. long, 7 mm. diameter, connate at the base, sometimes forking, often flattened and twisted, white, smooth or minutely pruinose, equal, hollow, cartilaginous; gills pallid, becoming brown or liver-coloured from the edge upwards, distant, equal, rather broad, rounded behind, adnate or almost free, interstices veined; spores white, narrow oval, inequilateral, or somewhat attenuated to the apiculus, apiculus prominent curved, 6–9 × 3–4 μ. Smell resembles that of *M. oreades*. On the ground, often with coarse rooting mycelium. Peradeniya, 6 April 1907; 30 June 1908; no. 6526, 1 May 1921 (Pl. III, fig. 15).

Marasmius leucophaeus (B. & Br.) Petch, in *Trans. Brit. mycol. Soc.* XXVII, 138; *A.* (*Collybia*) *leucophaeus* B. & Br., Fungi of Ceylon, no. 113; *Marasmius cornicolor* B. & Br., Fungi of Ceylon, no. 361.

Generally densely caespitose, sometimes scattered. Pileus usually 2–3·5 cm. diameter, plane, margin often repand, feebly striate in wet weather, centre even or depressed or umbilicate, or broadly convex, up to 5·5 cm. diameter, irregularly plicato-striate almost to the centre, deep purple brown, sometimes becoming paler outwards, often with an abruptly paler margin on small specimens and sometimes with a pale central spot over the stalk, appearing glabrous but covered with minute adpressed hairs, drying to ashy brown and minutely hoary, cartilaginous, thin, flesh purple brown; stalk up to 5 cm. long, 1–2 mm. diameter, dark purple brown when moist, ashy brown when dry, equal or slightly attenuated upwards, glabrous or minutely tomentose, often with a white tomentose ring at the apex, stuffed then hollow, often connate, base sometimes tomentose; gills narrow and crowded on small specimens, rather broad and subdistant on larger, adnate to the expanded apex of the stalk, pallid, becoming brown from above downwards, often retaining a pale edge; spores white, narrow oval, 6–10 × 3–4 μ. On the ground among dead leaves, etc. Common at Peradeniya (Pl. III, fig. 2).

A form occurs on the rough bark of living trees, e.g. jak, which appears very different, except during heavy rains, when it assumes the normal form. It is usually scattered, pileus at first hemispherical, then plane, up to 1 cm. diameter, grey, purple grey, or bluish grey, hoary with adpressed fibrils and arid-looking. This form is no. 3265, 13 December 1911; no. 4172, 11 October 1914; no. 4206, 25 October 1914, all on jak trees, Peradeniya.

The specimens in Thwaites's no. 204, noted by Berkeley and Broome as dull reddish purple to fuscous, and assigned to *M. Wynnei* var. *auroricolor* in Fungi of Ceylon, no. 353, are *M. leucophaeus*.

Marasmius rivulosus B. & Br., Fungi of Ceylon, no. 372.

Pileus campanulate to broadly convex, broadly umbonate, often depressed round the umbo, radially corrugated almost to the umbo, pale brown or tan, the colour varying with age and weather conditions, sometimes dark brown in the centre, ashy when dry, glabrous, about 4 cm. diameter but sometimes up to 10 cm., flesh white, thin except over the stalk; stalk up to 9 cm. long, 3 mm. diameter, base slightly expanded, longitudinally striate, at first smooth and shining, becoming finely white tomentose, pallid then purple brown, fibrous in wet weather, subcartilaginous when dry, stuffed then hollow, internally brown, often excentric; gills white or pinkish, becoming brown, moderately crowded, broad near the stalk, elsewhere narrow, arcuate, adnate or adnato-decurrent, connected by veins; spores white with a yellow tinge, varying from oval, 6 × 4 μ, to clavate or narrow oval with a thick excentric apiculus, 10–13 × 5 μ, in the same spore print. On decaying stumps or on a felted mycelium on chips of wood, scattered or clustered; stalks usually excentric when growing on stumps. Common at Peradeniya (Pl. II, fig. 2). Young specimens are sometimes purple brown at first, the colour changing to pale brown first in the centre, leaving the margin darker brown or brown in the furrows.

Specimens on a living palm stem (*Wallichia*), no. 6626, 12 June 1923, and on coir in orchid pots, no. 6762*a*, 19 June 1924, are more delicate and subpellucid, but otherwise the same.

Marasmius rufo-ochraceus Petch, n.sp.

Fasciculate or scattered. Pileus broadly convex to almost plane and irregularly undulating, up to 3·5 cm. diameter, red brown in the centre, becoming ochraceous or pallid outwards, or wholly ochraceous when dry, irregularly radially grooved, glabrous, thin, cartilaginous; stalk up to 3 cm. long, usually compressed, up to 3 mm. broad, cartilaginous, red brown, becoming paler upwards, glabrous or very minutely tomentose, stuffed then hollow; gills moderately distant, slightly ventricose, adnate, pallid becoming reddish brown, interstices strongly veined when old; spores white, clavate, 10–14 × 5–6 μ. On dead wood, Hakgala, no. 5583, 31 December 1917; no. 5603, December 1917; no. 6653, 7 September 1923.

Marasmius congregatus Mont., Guy. no. 307; *Cantharellus elegans* B. & Br., Fungi of Ceylon, no. 346; *Marasmius pellucidus* B. & Br., Fungi of Ceylon, no. 359.

In dense clusters among dead leaves or on rotten wood, with copious white rooting mycelium. Pileus frequently angularly campanulate at first, i.e. with a flat top and more or less perpendicular sides, then broadly campanulate, rarely plane, usually umbilicate, rather closely radially rivulose up to the centre, glabrous, translucent, papyraceous but tough, margin undulating, often repand, generally about 4 cm. diameter but sometimes up to 9 cm., white or yellowish; stalk up to 9 cm. long, 2–3 mm. diameter, rigid, cartilaginous, hollow, straight or twisted, glabrous, equal or expanding upwards, dark purple brown at the base, paler upwards and white at the apex, becoming entirely purple brown; gills white, usually narrow, about 1 mm. broad, but sometimes up to 4 mm., arcuate, forking, adnate to the expanded apex of the stalk, edge rather acute; spores white, clavate, 6–8 × 3 μ. Common at Peradeniya (Pl. III, fig. 1).

Berkeley and Broome's specimens of *Cantharellus elegans* were small examples; after the description they added '*Marasmius congregatus* Mont.'. Saccardo interpreted that as 'huc spectat *M. congregatus*'. Evidently Berkeley and Broome regarded the latter name as a synonym.

Marasmius crispatus B. & Br., Fungi of Ceylon, no. 357.

Clustered or scattered, among dead leaves. Pileus up to 7 cm. diameter, roadly convex, then plane, or repand and undulating, centre umbilicate, rregularly radially plicate almost to the centre, thin, tough, at first pallid rey brown, becoming buff on drying, glabrous, appearing cartilaginous vhen moist, but innately fibrillose when dry; stalk up to 9 cm. long, 4 mm. liameter, often compressed and up to 7 mm. broad, equal, expanding at he apex, cartilaginous, stuffed then hollow, dull, minutely powdered at irst, becoming minutely tomentose, clothed with white tomentum at the ase, with a mealy or minutely tomentose zone at the apex, coloured as the ileus at first, becoming purple brown from the base upwards; gills distant, rcuate, narrow, up to 4 mm. broad, sometimes forked, attenuated outvards, rounded behind and adnate to the expanded apex of the stalk, oloured as the pileus; spores white, oval, inequilateral, $8–10 \times 4\,\mu$. No. 239, Hakgala, 30 October 1914; no. 5235, Peradeniya, July 1917 (Pl. II, ig. 6).

In Herb. Peradeniya, there are two species in the specimens and paintings narked no. 766, *M. calvus*. That dated November 1868 is *M. crispatus*. Thwaites also sent specimens of the latter with *M. congregatus* in no. 38. t looks like a coarse *M. congregatus*.

Marasmius Caryotae (Berk.) Petch, *Ann. Perad.* iv, 403; *Heliomyces Caryotae* erk., *Hooker's Lond. J. Bot.* (1847), p. 491.

Pileus 1–2·5 cm. diameter, rarely up to 5 cm., campanulate, occasionally lepressed in the centre, then expanded with a recurved margin, deeply ulcate almost to the centre, glabrous, somewhat coriaceous, thin, pale ellow or greyish yellow, becoming ochraceous or pale brown when old, ed brown when dried; stalk 4–8 cm. long, 1–2 mm. diameter, white ecoming yellow, brown when old, rigid, hollow, almost glabrous, artilaginous, tomentose at the base; gills distant, broad, somewhat entricose, adnexed, rather thick, greyish yellow, brown when old; spores vhite, narrow oval or clavate, rather thick-walled, $20–25 \times 5\,\mu$. On the round among grass, usually in troops; common on the lawns at Peradeniya Pl. II, fig. 3).

arasmius hypochroides B. & Br., Fungi of Ceylon, no. 356.

Pileus up to 3 cm. diameter, broadly campanulate, margin irregular in xpanded specimens, rather pale red brown or olive brown, very thin but ough, not sulcate, cells of the pellicle crowned with close-set conical spines p to $10\,\mu$ long; stalk cartilaginous, hollow, fistulose, terete, 2 mm. iameter, or flattened, up to 4 mm. broad, equal or attenuated upwards or xpanded upwards, glabrous, red brown below, yellow brown above; gills hite, sometimes yellowish, distant, ventricose and up to 6 mm. broad in rge specimens, free, interstices veined; spores white, obliquely oval, $–13 \times 4–6\,\mu$. Among dead leaves, etc. Peradeniya, no. 4208, 25 October 914; no. 4244, 4 November 1914; no. 4372, 13 December 1914 (Pl. IV, gs. 10, 11).

Berkeley and Broome described the pileus as deeply sulcate; it has that appearance in the dried specimens, because of the tenuity of the pileus and the thickness of the gills, but fresh specimens are even, becoming sulcato-striate when collapsing.

Marasmius brunneostriatus Petch, n.sp.

Pileus campanulate, then almost plane, obtusely umbonate, up to 2 cm diameter, rufous, centre darker brown, margin paler, yellow brown radially sulcate, deeper coloured in the sulcae, minutely rugose, margin often repand; stalk up to 2 cm. long, 1 mm. diameter, expanded at the apex, cartilaginous, red brown, pruinose or minutely tomentose, base tomentose, arising from a thin stratum of white mycelium; gills pale ochraceous, distant, rather thick, narrow, equal, adnate; spores white, clavate or oval, 5–9 × 3–4 μ. On dead leaves and twigs. No. 4116, Hakgala, 29 September 1914 (Pl. II, fig. 7).

Marasmius fulviceps Berk., *Hooker's Lond. J. Bot.* (1847), p. 490; *Marasmius nummularius* B. & Br., Fungi of Ceylon, no. 351.

Pileus hemispherical, then broadly convex or almost plane, pale yellow or pale ochraceous, deeper ochraceous in the centre, or yellow brown, or sometimes deep brown, up to 1·5 cm. diameter, slightly wrinkled in the centre, radially striate elsewhere, glabrous; stalk up to 4 cm. long, 0·75 mm. diameter, blackish brown at the base, then red brown, yellow brown or white at the apex, cartilaginous, hispid, strigose at the base; gills crowded, narrow, white then pale ochraceous, adnate, the gill tissue continued from gill to gill round the apex of the stem; spores white, narrow oval, inequilateral, 10–12 × 4 μ. On decaying leaves and twigs, scattered or clustered, arising from a sheet of tawny mycelium, frequently with innate strands radiating from the base of the stalk. Peradeniya, no. 2903, 9 August 1909; no. 3505, 14 July 1912 (Pl. IV, fig. 6).

This species was sent from Ceylon by Gardner, with a figure. The Thwaites specimen referred to it by Berkeley and Broome (Thw. 807*) is *M. haematocephalus*.

Marasmius pallidorubens (B. & Br.) Petch, in *Trans. Brit. mycol. Soc.* XXVII, 138; *A.* (*Mycena*) *pallido-rubens* B. & Br., Fungi of Ceylon, no. 119.

Pileus campanulate or broadly convex, obtusely umbonate or depressed in the centre, membranous, translucent in wet weather, up to 4 cm. diameter, pale lavender, purplish brown in the centre, or in dry weather wholly vinous, strigose in the centre, elsewhere covered with scattered small scales with red brown points, plicato-sulcate almost to the centre, margin slightly serrate with brown points, flesh thin; stalk up to 3 cm. long, 2·5 mm. diameter, equal or slightly attenuated upwards, colour of the pileus, becoming red brown, covered with minute red brown points, hollow, subtranslucent; gills distant, ventricose, sinuato-adnate or adnato-decurrent, lavender becoming purplish red, edge clothed with red brown points, interstices veined; spores white, subglobose, or somewhat pyriform with an apical apiculus, 8–9 × 7–8 μ. The pileus and gills turn rather dark red when bruised; stains paper red. On dead wood. Peradeniya, no. 4277, 7 November 1914; no. 4382, 19 December 1914 (Pl. II, fig. 5).

After the description, Berkeley and Broome stated 'no. 937 is possibly the same species. Spores ·0003 by ·00025, echinulate'. The later statement is entered in Saccardo for *Mycena pallido-rubens*, but no. 937 in Herb. Peradeniya is a different species.

Marasmius proximus B. & Br., Fungi of Ceylon, no. 368; *M. obscuratus* B. & Br., Fungi of Ceylon, no. 370, not of Petch in *Ann. Perad.* VI, 56.

Pileus at first hemispherical, then expanded, plane, or depressed in the centre with a decurved margin, sometimes with a broad convex umbo and a depressed ring round it, feebly sulcate almost to the centre when fresh, pale ochraceous, or ashy and brownish in the centre, about 1 cm. diameter, rugose, minutely scurfy when dry; stalk about 1·5 cm. long, 0·5 mm. diameter, red brown at the base, yellow brown above, becoming black brown, cartilaginous, minutely pruinose or tomentose, sometimes with a white tuft of hyphae at the base, especially when growing on dead leaves; gills adnate or adnato-decurrent, pallid then pale brown, somewhat distant, rather ventricose. On dead leaves and twigs, sometimes accompanied by a white glabrous film of mycelium. Peradeniya, no. 3359, January 1912; no. 4152, 11 October 1914; no. 4205, 24 October 1914; no. 6553, 30 October 1922. Henaratgoda, nos. 5905, 5906, 25 June 1918. The pileus and gills sometimes turn red brown when dried (Pl. II, fig. 9).

Berkeley and Broome cited Thwaites no. 93 in part for this species. The remainder of no. 93 was assigned to *Naucoria furfuracea* P., together with Thwaites no. 1182. The latter has been shown to be *Flammula dilepis* B. & Br. (*Ann. Perad.* IV, 53), and the remainder of no. 93 is *Marasmius proximus*. *Naucoria furfuracea* should be deleted from the Ceylon list. The type of *Marasmius proximus* in Herb. Kew. is marked 93 and 398. No. 398 was described as *Omphalia delicia* B. & Br.; I have not examined the type.

The specimens and figure of Thwaites no. 804, which Berkeley and Broome placed as a variety of *Marasmius subcinereus*, are *M. proximus*. *M. mutabilis* was also Thwaites no. 804 (not 204) but the note 'pale dun colour when fresh' refers to *M. proximus*, not to it. *M. obscuratus* B. & Br. was based on small specimens of *M. proximus*.

Marasmius hirtellus B. & Br., Fungi of Ceylon, no. 382.

Pileus at first broadly convex, then almost plane or with a decurved margin, centre depressed, often with a broad convex umbo and a depressed ring round it, feebly radially sulcate towards the margin when fresh, ashy or ashy brown, usually with a pink or purple tinge when fresh, sometimes with a pale central spot, surface of innate matted fibrils, appearing smooth or minutely lacunose, about 1 cm. diameter; stalk up to 2·5 cm. long, 0·75 mm. diameter, cartilaginous, hispid, red brown, becoming black brown from the base upwards; gills rather crowded, narrow, adnate, white or pallid, becoming pale brown; spores white, narrow oval, somewhat clavate, 6–8 × 3 μ. On dead leaves and twigs, sometimes accompanied by a white glabrous film of mycelium. Fairly common at Peradeniya;

no. 4152, 11 October 1914; no. 4294, November 1914; no. 4300, November 1914; no. 5807, 27 October 1918; no. 5808, 28 October 1918 (Pl. II, fig. 4, and Pl. III, fig. 13).

Notwithstanding the figures, it is difficult to separate this species always from *M. proximus*. As a rule, the latter has broader and more distant gills.

Marasmius mutabilis B. & Br., Fungi of Ceylon, no. 367; *M. eximius* B. & Br., Fungi of Ceylon, no. 371.

Pileus infundibuliform, outer half sometimes almost plane, up to 2 cm. diameter, pale brown to reddish brown, glabrous, radially streaked with innate darker fibrils especially in the funnel, even, becoming radially sulcate, tough, thin, total height 1·5–2 cm.; stalk up to 1·4 cm. long, 1·5 mm. diameter, expanded upwards, at first purple brown at the base, white above, becoming dark purple brown, cartilaginous, minutely tomentose, usually with a rather compact whitish cushion at the base, faintly longitudinally striate; gills pallid, becoming brown when old, decurrent, rather distant, sometimes forked, arcuate, interstices veined; spores white, narrow oval, 8–9 × 4 μ. On dead wood and sticks. Peradeniya, no. 2298, 12 August 1906; no. 4289, 11 November 1914.

This was part of Thwaites no. 804, not 204 as stated in the Fungi of Ceylon. Berkeley and Broome said it was analogous to *Cantharellus rubidus*. They probably meant *C. rabidus*, Fungi of Ceylon, no. 349, and Saccardo printed it as *rabidus* in the note to *M. mutabilis*.

The type of *M. eximius* is a large specimen of *M. mutabilis*. It is infundibuliform, with broad decurrent gills, and innate radial dark fibrils on the pileus.

Marasmius senescens Petch, n.sp.

Pileus 1·5 cm. diameter, broadly convex, centre depressed, sometimes umbonate, even or irregularly plicato-sulcate, centre wrinkled, sordid grey with a paler margin, thin, centre slightly tomentose, elsewhere with a few scattered fibrils and here and there glistening particles; stalk 1 cm. long, 1·5 mm. diameter in the middle, expanding upwards, white or ashy, becoming black brown from the base upwards, compressed, striate, minutely longitudinally silky, cartilaginous; gills distant, adnate or adnato-decurrent, united behind over the stalk and separating, broad, slightly ventricose, rather thick, forked, pallid then brownish, interstices veined; spores white, narrow oval, somewhat pyriform, 7–10 × 3–4 μ. On the ground. No. 4306, Peradeniya, 15 November 1914.

Marasmius subcinereus B. & Br., Fungi of Ceylon, no. 369.

Pileus convex, then plane, slightly depressed, or umbilicate, sometimes infundibuliform in wet weather, sulcate almost to the centre, thin, dark green when young, then greenish grey or greenish white, almost black in the centre, or grey in the centre, white on the outer half, blotched green, becoming greenish brown or pale greyish brown when old, up to 1·5 cm. diameter; stalk up to 2·5 cm. high, 0·5 mm. diameter, rigid, dull, bluish black or black, pale towards the apex, hoary with minute white particles, solid, slightly thickened upwards, base either not thickened, or expanded into a disc up to 1·5 mm. diameter, or with a small tuft of white hyphae; gills white when young, then bluish or bluish grey, rather broad, distant, thick, adnate or slightly decurrent, interstices strongly veined; spores white, stellate, consisting of four broad-based triangular processes, each about 4 μ long and 3–4 μ diameter at the base, the distance from point to point being 8–12 μ. On young specimens, the pileus and gills turn blue to greenish black when bruised. On fallen branches and dead leaves. Peradeniya, not uncommon. No. 6627, 12 June 1923 (Pl. III, fig. 14).

A form with a pure white pileus and gills occurred on the trunks of living nutmeg trees at Peradeniya. Pileus up to 1·5 cm. broad, orbicular, convex or almost plane; stalk excentric, curved, up to 3 mm. long, 0·5 mm. diameter, expanding slightly upwards, greenish black, or black below, bluish above, pruinose; gills white, arcuate in convex pilei, ventricose in plane examples; colour change when bruised, and spores, as in the type. No. 6542, 22 September 1922; no. 6709, 25 December 1923 (Pl. III, fig. 5).

Marasmius corticigena B. & Br., Fungi of Ceylon, no. 388.

Pileus at first broadly convex, pale reddish brown in the centre, ashy or white towards the margin, margin even or faintly striate, minutely rugose; then expanded, up to 2·5 cm. diameter, broadly convex or plane, pale reddish brown, irregularly sulcato-striate over two-thirds the pileus, centre innately radially silky; stalk at first white, becoming brown from the base upwards, finally black brown, purple brown at the apex, expanding above, minutely tomentose, cartilaginous, base slightly tomentose, up to 2 cm. long, 0·8 mm. diameter in the middle; gills at first white, finally pale brown, subdistant, slightly ventricose, attenuated outwards, narrow, of four lengths, the longer adnate and united behind over the stalk, edges here and there tomentose with irregular, somewhat nodular hairs, up to 50 × 8 μ, sometimes encrusted at the apex, interstices veined; spores white, narrow oval or clavate, 8–12 × 3·5–4 μ. On living tree trunks. Peradeniya, no. 4370, 13 December 1914; no. 6486, 19 July 1922 (Pl. II, fig. 10).

Berkeley and Broome's specimens were quite immature, the pileus 3 mm. diameter, margin incurved, and the stalk 5 mm. long, brown to black. They show the tomentum on the gill edges.

Marasmius nivosus Berk., *Hooker's Lond. J. Bot.* (1856), p. 139.

Pileus at first broadly convex, then plane, even, or umbonate in the centre, becoming depressed or subumbilicate, or with a ring furrow surrounding the umbo, irregularly radially sulcate almost to the centre, white, pale ochraceous in the centre, or pale ochraceous with a pinkish tinge, becoming ashy on drying, coriaceous, 1–2 cm. diameter, margin irregularly crenate; stalk concolorous with the pileus, but purplish in very wet weather, up to 2 cm. long, 1 mm. diameter, equal, expanded slightly at the apex, cartilaginous, stuffed, glabrous or minutely pruinose; gills white, moderately crowded, arcuate, adnate or slightly decurrent. Among grass. Peradeniya, no. 6316, 20 November 1921; no. 6552, 30 October 1922; no. 6624, 12 June 1923 (Pl. III, fig. 12).

The fungus described above agrees with the specimens, Thwaites no. 932, in the Peradeniya herbarium, identified by Berkeley and Broome, but not altogether with the original description.

Marasmius stypinus B. & Br., Fungi of Ceylon, no. 389.

Pileus hemispherical, up to 8 mm. diameter, sometimes expanded, broadly convex, up to 1·5 cm. diameter, even or obtusely umbonate or sometimes depressed in the centre, expanded specimens feebly radially sulcate, white, slightly ochraceous in the centre, pruinose, or minutely tomentose on unexpanded specimens, becoming minutely sprinkled with brown points or almost glabrous, brown when old; stalk at first white, subtranslucent, rather stout, up to 1·5 cm. long, 0·6 mm. diameter, becoming red brown from the base upwards and diminishing to 0·4 mm. diameter, finally entirely blackish brown, cartilaginous, dull, minutely fibrillose or pruinose; gills distant, white, becoming brown when old, adnate, arcuate, or somewhat ventricose on expanded specimens, edge serrate; spores white, 10–12 × 4 μ, clavate, apiculus obtuse and sublateral, making the spore sometimes appear hooked at one end. On sticks and bamboos: remarkable for the persistence of its juvenile condition. Peradeniya, no. 2867, 4 July 1909; no. 2873, 18 July 1909; no. 2899, 5 August 1909; no. 4248, 4 November 1914 (Pl. IV, fig. 17).

Marasmius stypinoides Petch, n.sp.

Pileus white, greyish in the centre, broadly convex or almost plane, up to 5 mm. diameter, centre umbilicate, feebly radially sulcate, minutely rugose, margin crenate; stalk up to 8 mm. long, usually curved, 0·4 mm. diameter, at first white, turning black from the base upwards, shining, minutely powdered, longitudinally striate, expanded at the apex; gills white, crowded, rather ventricose, the longer adnate to the expanded apex of the stalk and separating as if collared, edge serrate. On dead bark on a fallen tree trunk. Hakgala, no. 4029, April 1914.

No. 4114, on a dead leaf, Hakgala, September 1914, is a similar species, but is umbonate. Unfortunately I have seen only one example.

Marasmius cineraceus Petch, n.sp.

Pileus infundibuliform, margin decurved, feebly radially sulcate, 9 mm. diameter, ashy, smooth, with a surface layer of interwoven hyphae; stalk black, with a white pruina, cartilaginous, 1 cm. long, 0·75 mm. diameter, equal, arising from a small circular glabrous disc; gills moderately crowded, white, sigmoid, broad, adnato-decurrent, margin crenate, interstices veined. On a dead leaf. Peradeniya, no. 5589, October 1918.

Marasmius Leveillianus (Berk.) Pat., *Bull. Soc. Mycol. Fr.* XXXIII (1917), 55; *Heliomyces Leveillianus* Berk., Decades of Fungi, no. 158; *Marasmius umbraculum* B. & Br., Fungi of Ceylon, no. 365.

Pileus up to 3·5 cm. diameter, at first hemispherical, becoming plane, umbonate, depressed round the umbo, radially sulcate, rigid when dry, ep red brown, glabrous; stalk up to 8 cm. long, 0·5 mm. diameter, black own, equal, expanding suddenly at the apex, rigid, horny, glabrous, llow; gills distant, usually all the same length, free, truncate behind, hite becoming yellow, margin sometimes red brown, interstices sometimes ined; spores white or faintly yellowish, narrow oval, 8–10 × 3–4 μ. regarious, on dead stumps, bamboos, etc., rather common. Peradeniya, June 1906; 15 July 1907; 15 July 1909, etc. Wariapolla, 6 December 06 (Pl. IV, fig. 2).

arasmius atrorubens Berk., *Hooker's Lond. J. Bot.* 1, 188.

Pileus campanulate at first, hispid with setae like those of the stalk but iorter, then broadly convex and glabrous or nearly so, 1 cm. diameter, eep red brown, somewhat mottled, margin faintly striate when fresh, rooved when dry, cells of the pellicle covered with short spines up to 5 μ ng; stalk up to 4·5 cm. long, 0·5 mm. diameter, horny, rigid, red brown, rown when dry, hispid with brown, rigid acute setae, up to 120 μ long, -4 μ diameter, apparently persistent; gills crowded, pallid then pale chraceous, free. In groups among dead leaves, arising from yellowish rigose patches of mycelium. Peradeniya, no. 5176, 10 June 1917.

The above description refers to Ceylon specimens which appear to fit erkeley's description. They have not yet been compared with the type om Cuba. The Ceylon specimens attributed by Berkeley and Broome to *ollybia stupparia* B. & C. are this species.

arasmius florideus B. & Br., Fungi of Ceylon, no. 380.

Pileus campanulate or conico-campanulate, then expanded and margin epand, apex sometimes flattened, up to 2 cm. diameter, red brown, dark the centre, becoming paler outwards, plicato-striate almost to the entre, thin but tough; stalk up to 5·5 cm. high, 1 mm. diameter, glabrous, orny, red brown to yellow brown below, pale at the apex, becoming lackish brown from the base upwards, base strigose; gills cream-coloured yellowish, edge red brown, ventricose, adnate, moderately distant, of our lengths. Among dead leaves. Peradeniya, no. 4207, 25 October 1914 Pl. IV, fig. 3).

arasmius confertus B. & Br., Fungi of Ceylon, no. 352; *M. chondripes* B. & r., Fungi of Ceylon, no. 364; *M. hemibaphus* B. & Br., Fungi of Ceylon, o. 379.

Pileus hemispherical, then expanded, broadly convex or almost plane, eddish brown in the centre, elsewhere tawny or golden brown, centre even r slightly depressed, margin feebly sulcate, usually about 1·5 cm. diameter, xceptionally 3 cm., membranous, the cells of the pellicle bearing close-et conical spines up to 6 μ long; stalk up to 3·5 cm. long, 0·5 mm. diameter, gid, horny, shining, often flexuose, yellow brown, red brown at the base, hite at the apex at first, attached by a tuft of tawny fibrils; gills crowded, ellow or yellow brown, shortly adnate, attenuated outwards, narrow; ores white, clavate, 11–13 × 3 μ. Scattered or clustered, on the ground among dead leaves and twigs. Peradeniya, no. 2408, 14 June 1907; no. 2870, 16 July 1909. Henaratgoda, no. 5904, 25 June 1918.

Berkeley and Broome gave the spores as subglobose, 6 μ diameter. Their type specimen bears a few minutely warted globose spores about 4 μ diameter, no doubt intrusive *Penicillium* or *Aspergillus*.

Marasmius hemibaphus was described as umbonate and sulcate, but the sulcae are chiefly the result of drying, and the pileus is not umbonate, but darker in the centre than on the outer half, a fact which perhaps suggested the name. There was no painting of this species. Thwaites no. 204, from which it was described, was a mixture, part of which was *Collybia leucophaea*, and the painting, no. 204, is of the latter species. *Marasmius chondripes*, also part of Thwaites no. 204, is a young *M. confertus*.

Marasmius haematocephalus Mont., *Syll. Crypt.* no. 351.

Pileus conico-campanulate or hemispherical, up to 7 mm. diameter, sometimes repand and up to 2 cm. diameter, plicato-sulcate almost to the centre, membranous, purple red or bluish purple or red brown; stalk hair-like, flexuose, usually 3–4 cm. long, 0·25–0·5 mm. diameter, horny, shining, black brown below, pale brown above, with a small tuft of white hyphae at the base; gills white, often tinged purple like the under side of the pileus, often with a red edge, free but close to the stalk or adnate to the swollen apex of the stalk, few, distant, ventricose, interstices veined when old; spores white, clavate (like an Indian club), 16–22 × 3–4 μ. On decaying leaves and twigs, gregarious. Common in mid and low country (Pl. IV, fig. 4).

No. 3510, Peradeniya, 21 July 1912, was dark greenish grey or olive, but otherwise did not differ from the typical purple red form.

Marasmius helvolus var. *brunneolus* B. & Br., Fungi of Ceylon, no. 375.

Pileus conico-campanulate or hemispherical, about 1 cm. diameter, 8 mm. high, brown in the centre, becoming yellow brown outwards, membranous, plicato-sulcate nearly to the centre or half-way, minutely velvety, the spines on the cells of the pellicle tending to be arranged in dots; stalk up to 4·5 cm. long, 0·5 mm. diameter, equal, glabrous, shining, at first pellucid, becoming brown from the base upwards, with a spreading tuft of white or tawny hyphae at the base; gills distant (about twelve), white, free, ventricose; spores white, narrow clavate, 16–19 × 4 μ. On dead branches and leaves. Peradeniya, no. 2904, 8 August 1909; no. 4204, 23 October 1914 (Pl. IV, fig. 13).

The specimens described above agree with those of Thwaites no. 752 at Kew and Peradeniya. Berkeley and Broome stated that the pileus and gills were brownish, but there was no figure. *M. helvolus* Berk., type, was described from Cuba.

Marasmius coniatus B. & Br., Fungi of Ceylon, no. 358.

Pileus conico-campanulate, up to 4 mm. diameter and 3 mm. high, plicato-sulcate, olive brown in the centre, becoming brownish buff outwards, membranous, sprinkled with minute glistening particles, especially in the furrows, cells of the pellicle bearing close-set cylindrical spines up to 6 μ high; stalk black (brown when dry), shining, hair-like, up to 2·5 cm. long, 0·2 mm. diameter, pellucid at the apex, arising from a thin white layer of mycelium; gills free, distant, ascending, pallid, edge brown. On dead leaves and twigs. Peradeniya, no. 4221, 25 October 1914 (Pl. IV, fig. 1).

Marasmius subconiatus Petch, n.sp.

Pileus up to 5 mm. diameter, broadly convex, centre umbilicate, plicato-sulcate to the centre, dull reddish brown, sprinkled with minute hyaline particles, cells of the pellicle crowned with close-set conical spines; stalk up to 6 mm. long, stout, 0·4 mm. diameter, black brown, glabrous, cartilaginous; gills numerous (14), distant, cream-coloured, broad, lower edge straight or somewhat ventricose, broadly adnate. On bamboo. Peradeniya, no. 4295, 12 November 1914.

Looks like a small stout form of *M. coniatus*, but differs in the number and attachment of the gills, and the absence of a film of mycelium on the substratum.

Marasmius semipellucidus B. & Br., Fungi of Ceylon, no. 360.

Pileus conico-campanulate, sometimes expanding to almost plane, sometimes with a recurved margin, up to 1 cm. diameter, membranous, plicato-sulcate almost to the centre, pinkish brown; stalk usually about 3 cm. long, but sometimes up to 8·5 cm., 0·5 mm. diameter, at first light brown, becoming almost black at the base, horny, smooth, the base surrounded by a small cushion of hyphae; gills white, free, ventricose, distant (usually 12 to 16), sometimes with a brown or reddish edge; spores white, clavate, 14–22 × 4–5 μ. The whole pileus and gills have a pinkish tinge when moist. On decaying leaves and twigs. Peradeniya, nos. 2437, 2438, 16 June 1907; no. 4287, 9 November 1914; no. 4315, 20 November 1914 (Pl. IV, fig. 8).

Berkeley and Broome described the stalk as 'toto striato', but that is not evident on fresh specimens. They also stated that it is distinguished by the upper part of the stem being pale and pellucid, a feature which is common to all species of this class in their earlier stage.

Marasmius lateritius Petch, n.sp.

Pileus convex or conico-convex, even or obtusely umbonate, up to 6 mm. diameter, pinkish red or brick red, darker when old, often mottled with yellowish patches, margin not striate or plicate (except when old); stalk up to 2·5 cm. high, 1 mm. diameter, at first white, then yellow brown from the base upwards, horny, minutely hispid, strigose at the base; gills white, then yellow, not distant, rounded or truncate behind, attenuated outwards, free but close to the stem; spores white, clavate, 10–13 × 3–4 μ. On the ground among dead leaves. Peradeniya, no. 4375, 15–16 December 1914 (Pl. IV, fig. 5).

Marasmius albocapitatus Petch, n.sp.

Pileus pure white, hemispherical, up to 4 mm. diameter, minutely umbilicate, feebly sulcate; stalk up to 1·5 cm. long, 0·2 mm. diameter, black brown, hair-like; gills white, narrow, ascending, adnate, edge obtuse; spores white, narrow oval, 8–10 × 3 μ. The pileus and stalk bear long erect spreading hairs, not visible to the naked eye, yellow brown by transmitted light, acute, tapering, about 4 μ diameter below, up to 0·35 mm. long, and the stalk bears in addition minute irregular thick-walled hairs, usually curved, up to 30 × 6 μ. Cystidia on the edge of the gills flask-shaped, up to 34 μ high, 12 μ diameter below. On dead leaves. Hakgala, no. 4127, September 1914; no. 5294, April 1917 (Pl. IV, fig. 15).

Marasmius micraster Petch, n.sp.

Pileus 3 mm. diameter, almost plane when moist, campanulate and radially plicate when dry, black brown to yellow brown, paler in the furrows, membranous, centre depressed and wrinkled, cells of the pellicle globose, about 16 μ diameter, strongly verrucose on the outer half with yellow brown warts, pileus appearing minutely pruinose when magnified; stalk hair-like, black brown, glabrous, shining, about 1 cm. long, 0·2 mm. diameter; gills few (about ten), white or cream, ventricose, free but close to the stem, edge bears flask-shaped cystidia, up to 16 μ high, with a truncate apex; spores white, oval, one end acute, 10–12 × 4–5 μ. On decaying twigs. Peradeniya, no. 4195, 21 October 1914.

Marasmius Thwaitesii B. & Br., Fungi of Ceylon, no. 383.

Pileus in dry weather cylindrical, 2–2·5 mm. diameter, 3 mm. high, dark brown, covered with spiny clusters of hairs and plicate in longitudinal ridges; when moist, broadly campanulate or almost plane, up to 6 mm. diameter, plicato-sulcate, blackish brown in the centre, somewhat lighter brown along the tops of the ridges and pale brown in the furrows; the centre and the dark ridges retain the hair clusters for some time, but they ultimately disappear, first from the ridges and then from the centre; the ridges are produced beyond the margin of the pileus by a spinous tuft of hairs; stalk up to 2·5 cm. long, 0·25 mm. diameter, rigid, dark brown, almost white at the apex, rough; gills about twenty, moderately distant, yellowish white, edge brown distally, narrow, united behind by a very narrow collar. On sticks and decaying palm stems. Frequent at Peradeniya. No. 4353, 1 December 1914 (Pl. IV, fig. 9).

Marasmius gordipes Sacc. & Paol., *Mycetes Malacc.* p. 6, Pl. V, fig. 2.

Pileus membranous, broadly convex, with or without an acute black umbo at the base of the umbilicus, sulcate to the centre, 3–5 mm. diameter, red brown; stalk filiform, very long, up to 13 *centimetres* long, 0·3 mm. diameter, somewhat rigid, striate when dry, glabrous, shining, blackish brown, insititious; gills distant (up to 12), yellowish or ochraceous, sometimes with a reddish edge, comparatively broad, united behind into a collar which may fit closely round the stalk or be widely separated from it and shallow; spores white, narrow oval, somewhat clavate, 8–12 × 4–5 μ On decaying twigs and leaves on the ground. Peradeniya, no. 2902, 9 August 1909; no. 4235, 1 November 1914. Not a Horsehair Blight (Pl. IV, fig. 7).

Marasmius tubulatus Petch, n.sp.

Pileus cylindrico-campanulate, deeply umbilicate, radially sulcate to the centre, pruinose, membranous, up to 8 mm. diameter, pallid then brownish grey with a pale margin and a black spot at the base of the umbilicus; stalk up to 2 cm. long, 0·3 mm. diameter, blackish brown, horny, glabrous, shining, finely longitudinally striate; gills distant, white then pallid with a brownish grey edge, subtriangular, united behind into a tube, free from the stem, which descends almost to the level of the margin of the pileus, free edges arched; spores white, oval or clavate, 7–9 × 3–4 μ. On dead leaves and twigs. Peradeniya, no. 4243, 1 November 1914; also 18 July 1909 (Pl. III, fig. 9).

Marasmius griseoviolaceus Petch, n.sp.

Pileus campanulate, up to 1 cm. diameter, umbilicate, plicato-sulcate, membranous, greyish or brownish violet or purplish brown, or almost black in the centre and blackish grey elsewhere, with or without a minute umbo at the base of the umbilicus, feebly rugose with minute black elevations when fresh; stalk up to 7 cm. long, 0·5 mm. diameter, attenuated upwards, rigid, horny, black, shining; gills white, distant (up to 14), arcuate or triangular, edge coloured as the pileus, united behind into a collar round the stem; spores white, oval, inequilateral, 8–11 × 6 μ. Has a black repent rhizomorphic mycelium, 0·25 mm. diameter, and arises either from the rhizomorphs or from the host tissue. Peradeniya, at the base of clumps of Giant Bamboo (*Dendrocalamus giganteus*), no. 4238, 1 November 1914; on dead leaves, no. 4213, 25 October 1914 (Pl. IV, fig. 14).

Marasmius rotalis B. & Br., Fungi of Ceylon, no. 387; Petch in *Ann. Perad.* VI, 50.

Pileus 4–6 mm. diameter, hemispherical, umbilicate, generally with a minute umbo at the base of the umbilicus, minutely rugose, radially sulcate with from eight to eighteen grooves, yellow brown, greyish brown or ashy, membranous; stalk 2–4 cm. long, 0·2–0·5 mm. diameter, black, horny, filiform, shining; gills white or yellowish, distant, rather broad, lower edge almost horizontal, united behind into a collar round the stalk; spores white, narrow oval, 8–12 × 3–4 μ. On dead leaves and twigs on the ground, with black, glabrous, cylindric rhizomorphic mycelium about 0·1 mm. diameter. Peradeniya and Hakgala, frequent.

The following colour varieties have been noted. Pileus orange red, gills orange, stalk brown, Hakgala, no. 3725, 23 May 1913. Pileus, gills and stalk orange, Hakgala, no. 4137, September 1914. Pileus greyish fawn, gills white, stalk black brown, Hakgala, no. 4129, 29 September 1914. Pileus olive brown, Hakgala, no. 4138, September 1914. Pileus white, umbo black and prominent, Hakgala, no. 5587, December 1917.

Marasmius equicrinis Muell., *J. Linn. Soc.* XVIII, 383; Petch in *Ann. Perad.* VI, 43.

Mycelium rhizomorphic, black, smooth, glabrous, cylindrical, about 0·1 mm. diameter, overrunning leaves and stems of living shrubs and trees

(Horse-hair Blight). Pilei arising laterally from the aerial mycelium, or from dead leaves and twigs on the ground.

Pileus up to 8 mm. diameter, hemispherical, often irregularly repand, umbilicate, membranous, radially sulcate, yellow brown to red brown, with a minute black umbo; stalk 2–10 mm. long, 0·1 mm. diameter, filiform, black, glabrous; gills few (up to eight), white, then cream or yellowish, distant, attenuated behind, united into a collar round the apex of the stalk; spores white, narrow oval, inequilateral, or clavate, 10–14 × 4 μ. Cells of the pellicle (as in *M. rotalis*) crowned with close-set, blunt spines. Common in the wet low-country of Ceylon, up to an elevation of 1600 ft. (Pl. III, fig. 8).

Marasmius rigidichorda Petch, n.sp.; *M. obscuratus* Petch, *non* B. & Br., in *Ann. Perad.* VI, 56.

Mycelium rhizomorphic, dull black, rigid, 0·4–0·6 mm. diameter, overrunning the branches of jungle shrubs. Pilei produced on the aerial mycelium and on the dead tissues of the host plant.

Pileus hemispherical, umbilicate, with margin narrowly decurved, sometimes infundibuliform, plicato-sulcate almost to the centre, coriaceous, dark brown in the centre, elsewhere reddish brown, becoming ashy or brownish white, streaked with brown fibrils and points, 1 cm. diameter; stalk up to 1 cm. long, 1 mm. diameter, blackish brown, cartilaginous, rough with minute fascicles of hairs; gills distant, narrow, arched, adnate to a disc at the apex of the stalk, reddish, then creamy white, rather thick, interstices sometimes veined; spores white, narrow oval, inequilateral, sometimes curved at one end, 8–10 × 3–4 μ. In wet low country jungles from 400 to 2000 ft. elevation. Hapugastenne, nos. 2991, 3226, October 1909. This might be known as Elephant Hair Blight.

In *Ann. Perad.* VI, 56, this species was assigned to *M. obscuratus* B. & Br. a re-examination of the type of the latter has shown that it consists of rather young examples of *M. proximus*, and is different from the present species.

Marasmius ascendens Petch, n.sp.

Mycelium rhizomorphic, black, glabrous, angular, flattened and twisted, 0·2–0·4 mm. broad, overrunning the bark of living trees up to a height of 40 or 50 ft. and forming a tangle among the upper branches. Pilei formed on fallen affected branches.

Pileus ashy to purple grey, up to 1 cm. diameter, convex, then plane or repand, sometimes umbonate, membranous, tough, surface minutely rugose; stalk up to 1 cm. long, filiform, 0·2 mm. diameter, or flattened, 0·4 mm. broad, glabrous, horny, black below, purple brown above; gills distant, adnate or very slightly decurrent, very narrow. Hakgala, nos. 4559, 4643, March 1915.

Marasmius actinophorus B. & Br., Fungi of Ceylon, no. 385; *M. coronatus* Petch, *Ann. Perad.* VI, 58.

Mycelium rhizomorphic, about 0·5 mm. diameter, dark brown, closely covered with adpressed white hairs, which are simple, up to 0·6 mm. long, 4–6 μ diameter, septate, thick walled, overrunning shrubs and tree trunks up to a height of about 4 ft. Pilei produced terminally on the mycelium and also on dead branches or the bark of living trees.

Pileus hemispherical, or broadly convex or almost plane, about 1 cm. diameter; centre black or dark brown, depressed, usually with a minute conical umbo, clothed with coarse radially arranged fibrils which project in a fringe beyond the central area, elsewhere, brownish white with radial ridges of coarse hairs which project over the margin; hairs on the pileus, 3·5–4 μ diameter, smooth, simple, thick-walled, flexuose below, separate at the base, cohering above in pointed tapering fascicles up to 0·5 mm. long; stalk 4–7 cm. long, 0·25–0·5 mm. diameter, black, dull, not horny, equal, thickly clothed with adpressed white hairs; gills white, crowded, free, slightly ventricose; spores white, narrow oval, slightly inequilateral, 7–9 × 4–5 μ. Common at Hakgala (Pl. IV, fig. 16).

Marasmius Hakgalensis Petch, n.sp.

Pileus hemispherical, or broadly campanulate, up to 4 mm. diameter, purple grey or ashy brown, darker in the centre, membranous, minutely rugose, even, not umbilicate or umbonate, feebly striate at the margin; stalk up to 3 cm. long, 0·5 mm. diameter, purple brown or black, equal, horny, clothed with scattered, rigid, spreading, white hairs; gills moderately distant, adnate, white, or pallid with a white edge. On dead leaves and twigs on the ground, associated with black, angular, rhizomorphic mycelium, at first setulose like the stalk, becoming glabrous. Hakgala, common in jungle belts (Pl. IV, fig. 12).

Var. **denudata** Petch. Stalk glabrous; gills more crowded, purplish (Pl. IV, fig. 12 left).

Marasmius Paspali Petch, n.sp.

Pileus hemispherical, not umbonate or depressed in the centre, bluish grey, feebly sulcate, pruinose, 2·5 mm. diameter; stalk excentric, blackish, white pruinose, fibrillose, cartilaginous, slightly attenuated upwards, 2 mm. long, 0·2 mm. diameter; gills ochraceous, rather broad, few (three or four, with short intermediates), adnate, interstices veined. On leaf-sheaths of *Paspalum dilatatum*, apparently parasitic. Old Peradeniya, no. 6531, 12 August 1922 (Pl. III, fig. 11).

Marasmius Campanella Holtermann, *Myk. Unters. aus den Tropen* (1898), p. 105; '*Cantharellus capensis*' (in part) in Fungi of Ceylon, no. 350. Brown variety, *Marasmius rufescens* B. & Br., Fungi of Ceylon, no. 394; *Xerotus tener* B. & Br., Fungi of Ceylon, no. 426.

Pileus circular, orbicular, or reniform, up to 4 cm. diameter, but usually about 2 cm., campanulate, then convex, edge often recurved, sulcate over the gills and veins, depressed over the stalk, glabrous, slightly rugose, membranous, tough, not gelatinous, white, grey or purple grey or greyish brown, blackening slightly when old, sometimes rufous, rarely orange; stalk short, excentric, curved, sometimes central, 3–4 mm. long, 1 mm. diameter, solid, at first the same colour as the pileus and clothed with minute fibrils, then black and glabrous, expanding at the apex, swollen at the base; gills few, usually narrow but sometimes thin and broad (up to 3 mm.), distant, often forked, united by strong veins when old, adnate to the swollen apex of the stalk, the whole lower surface of the pileus slightly paler than the upper; spores white, oblong oval, 7–12 × 5–6 μ. On dead branches, stems of lianes, etc. Generally distributed and frequent (Pl. II, fig. 14; Pl. III, fig. 6; Pl. IV, fig. 18).

This species was discussed in *Ann. Perad.* IV, 404–6 (1910), where it was recorded that Berkeley and Broome's Ceylon specimens of *Cantharellus capensis* contained at least two species, one membranous and stalked, the other gelatinous and usually sessile, which answered to Holtermann's *Marasmius Campanella* and *Favolaschia bispora* respectively, and it was pointed out that Thwaites's figure, no. 90, cited for *Cantharellus capensis* was *Favolaschia bispora*. The latter species was not described until 1912, when it appeared in Sacc. *Syll. Fung.* XXI, 358 as *Laschia bispora* (Holt.) Sacc. & Trav. with a spore measurement the same as *Marasmius Campanella* and certainly wrong for both. These species are said to have been collected in Java, but Holtermann did not give the locality, and as he worked for some time at Peradeniya, and the two formerly occurred in quantity near the entrance to the Botanic Gardens where they could scarcely have been overlooked by a mycologist, it would seem probable that he may have collected them in Ceylon. In *Mycol. Notes*, no. 58 (1919), 815, C. G. Lloyd stated that, from the figure, *M. Campanella* was probably in part *Campanella cucullata* (Jungh.) Lloyd, and in no. 70 (1923), 1225, he recorded that Berkeley's Ceylon determinations of *Cantharellus capensis* were *Campanella cucullata*. Both these statements no doubt refer to *Laschia bispora*, but as its spores are not globose it cannot be *Campanella*.

Marasmius rufo-aurantiacus Petch, n.sp.

Pileus at first galeate, then expanded, broadly campanulate or almost plane, orbicular, up to 2 cm. diameter, red brown at first, becoming brownish fawn or buff, smooth, thin, membranous, large specimens sulcate; stalk excentric, white or brownish, minutely powdered or tomentose, tomentose at the base, 2–3 mm. long, 0·3 mm. diameter; lower surface and gills bright orange, fading to fawn; gills adnate, arched, rather broad, edge thick, interstices veined, spotted with clusters of cylindrico-clavate cystidia crowned with red brown deposits; spores white, broadly clavate, slightly curved at the apex, 8–12 × 4 μ. On dead twigs and bark. Hakgala, no. 4027, April 1912; no. 4028, March 1914; no. 4130, September 1914 (Pl. II, fig. 13).

Marasmius porphyreticus Petch, n.sp.

Pileus usually galeate, then almost plane, orbicular, stalk excentric, almost lateral, or convex, almost centrally stalked, pale or dark purple brown, rugose, plicato-sulcate, up to 2 cm. diameter; stalk short, 1 mm. diameter, expanding upwards, purple brown, glabrous; lower surface and gills pale purple brown; gills distant, adnate, about six reaching the top of the stalk with shorter intermediate, usually forked, edge thick; spores white, narrow oval, 7–11 × 3–4 μ. On dead sticks. Hakgala, no. 4126, 28 September 1914 (Pl. II, fig. 11).

Marasmius alliarius Petch, n.sp.

Pileus orbicular, up to 2 cm. broad, membranous, broadly convex, smooth, red brown, sulcate towards the margin when fresh; stalk excentric, very short, expanding upwards, longitudinally silky, white; gills cream-coloured, of three lengths, the longer adnate, arched, distant, broad, interstices veined; spores white, oval, somewhat inequilateral, 7–10 × 4–5 μ. Strong smell of garlic when bruised. On dead wood and sticks. Hakgala, no. 3539, April 1912; no. 3910, January 1914; no. 4060, April 1914; no. 4131, September 1914; no. 5585, December 1917 (Pl. II, fig. 12).

Marasmius ignobilis B. & Br., Fungi of Ceylon, no. 390.

Pileus orbicular, convex, pale fawn-coloured, thin, minutely rugose, up to 8 mm. diameter; stalk white, excentric, curved, up to 1·5 mm. long, 0·5 mm. diameter, pruinose or subtomentose; lower surface white; gills distant, adnate, edge arcuate or straight, sometimes forked, connected by strong veins. Old specimens ochraceous, the stalk blackening at the base. Strong smell of garlic when fresh. On dead twigs on the ground. Henaratgoda, no. 5346, 12 September 1917.

No number or date was cited by Berkeley and Broome for this species, which was said to have occurred on dead wood in the south of the island. In *Ann. Perad.* IV, 391, I referred it to *Oudemansiella subaurantiaca*, basing that opinion on Thwaites no. 100, Peradeniya, which was cited under *Marasmius ignobilis* as a variety. There is, however, in Herb. Kew., in the cover of *M. epochnous*, Thwaites no. 683, a specimen on twigs from the south of the island, marked *M. ignobilis*, which answers to Berkeley and Broome's description and that given above.

Marasmius purpureo-albus Petch, n.sp.

Pileus usually orbicular, up to 3 cm. diameter, stalk excentric, convex, then repand, sometimes centrally stalked, broadly convex, at first pale purple, becoming white or pale ochraceous, depressed or umbilicate over the stalk, irregularly plicate, glabrous; stalk white, stout, up to 1 cm. long, 2 mm. diameter in the middle, equal or expanding upwards, with a ring of tomentum at the base; gills distant, ventricose, the longer sinuato-adnate, white or pallid; spores white, narrow oval, inequilateral, 10–14 × 5–6 μ. On a decaying banana stem, no. 5303, Peradeniya, August 1917. On bark scales on living *Lagerstroemia*, no. 6541, Peradeniya, 21 September 1922; on fallen twigs of *Lagerstroemia*, no. 6557, Peradeniya, 5 November 1922 (Pl. III, figs. 3, 10).

Marasmius Amomi Petch, n.sp.

Pileus at first convex, purple brown, tomentose with spongy tomentum and a few adpressed white hairs, then broadly convex, plane, or irregularly repand, up to 5 cm. diameter, irregularly radially plicate, purplish brown at the base, elsewhere pale ochraceous, sparsely covered with innate radiating fascicles of purple brown fibrils, matted silky between; stalk excentric, white, glabrous, expanding upwards, up to 1 cm. long, 1·5 mm. diameter, with a basal tuft or cushion of orange hyphae; gills white or pallid with a purple tinge, distant, adnate, forked, the edge irregularly crisped in repand specimens, interstices veined. On dead stems of *Amomum*. Hakgala, no. 4652, April 1915.

Marasmius Calami Petch, n.sp.

Pileus up to 2 cm. diameter, membranous, broadly convex, strongly sulcate over the gills and irregularly bullate between, white with a greenish tinge, margin when fresh fimbriate with clavate hairs, 3 μ diameter below, 4–8 μ above; stalk excentric, curved, connate at the base, up to 1 cm. long, 1 mm. diameter, equal, expanded at apex, white, longitudinally silky striate; gills white, rather thick, the longer distant, adnato-decurrent, often forked, with shorter intermediate gills, connected by strong veins; spores white, clavate, 13–16 × 4–5 μ. On a dead stem of *Calamus*. Peradeniya, no. 6708, 25 December 1923.

Marasmius pulcher (B. & Br.) Petch, *Ann. Perad.* IX, 21; *Cyphella pulchra* B. & Br., Fungi of Ceylon, no. 665.

Mycelium without anker cells, running in stout white cords over the stems and leaves of living shrubs; pilei produced at the edge of the cords or on diffuse patches of mycelium on dead leaves and bark. Pilei sessile, resupinate or laterally attached, sometimes shortly stalked, reniform or orbicular, sometimes lobed, white, becoming pale ochraceous, thin, tomentose, sulcate over the gills, up to 7 mm. diameter; gills radiating from an excentric point, few or absent on small examples, crowded and of medium breadth on the larger; spores white, cymbiform, 6–8 × 4 μ. Mycelium (Thread Blight) common in the wet low-country. No. 5269, from Hakgala, may be a different species; it was found running over and among dead bark scales on a dead standing tree trunk and its spores were measured as oval or subglobose, 4–5 × 3 μ (Pl. III, fig. 4).

SPECIES DUBIAE ET EXCLUDENDAE

Marasmius concolor B. & C.

The Ceylon specimens attributed to this species (Thwaites no. 685) are *Crepidotus melleus* (B. & Br.) Petch (*Aschersonia mellea* B. & Br.).

Marasmius epochnous B. & Br., Fungi of Ceylon, no. 393; '*M. epochnous* B. & C.' (in error).

This species was said to be white. The pilei in the type are gregarious, up to 3 mm. diameter, orbicular, convex, tomentose; stalk excentric, almost lateral, curved, up to 0·5 mm. long, 0·15 mm. diameter, each arising from a circular patch of radiating hyphae; gills broad, adnate, distant. The pileus and gills are very friable, and it does not seem a good *Marasmius*. It was collected in the south of the island and has not been matched by recent specimens. It probably grew on the under surface of a branch as, in the herbarium specimen, the stalks curve towards the substratum, so that the lower surface of the pileus is uppermost.

Marasmius (*Mycena*) *galericula* Ces.

This species was collected by Beccari in the Botanic Gardens, Peradeniya, and was described by Cesati as 'subfuniculate, lignicolous; stalk three inches long, filiform, horny, rigid, brown, flexuose (not twisted), smooth, scarcely pulverulent or pruinose, but opaque; pileus campanulate, one inch diameter, colour when dry lurid, with the appearance of an agaric of the series *Mycenae rigidipes*, collapsed when old. From the development of the stalk I am doubtful as regards *Marasmius*.' *Mycenae rigidipes* is Fries's section of *Mycena* which includes *Mycena galericulata*, and from Cesati's choice of a name it might be surmised that he saw some resemblance to that species. I am unable to suggest what it may have been.

Marasmius inustus B. & Br., Fungi of Ceylon, no. 384.

The type resembles rather small specimens of *M. Campanella*, somewhat thicker than usual, and stained black here and there, as though it changed colour when bruised. They have not been matched by fresh specimens and may be a distinct species. They are not gelatinous.

Marasmius prasinus B. & Br., Fungi of Ceylon, no. 373.

Berkeley and Broome described this as pileus 3 cm. across, of a delicate greyish green, somewhat fleshy, glabrous, margin irregular, sulcate, flesh white; stalk 3 cm. long, 1·5 mm. thick; gills narrow, arcuate, decurrent, ochraceous; on dead twigs, etc. No Thwaites number, locality or date was cited, and there was no painting. The type in Herb. Kew. consists of a single pileus pressed flat, about 4 cm. diameter, extremely thin. It bears on the lower surface radial bands of loose tissue, bounded by denser lines, and examination shows that the gills have been cut away, leaving the trama bounded by their bases. Pieces of the gill remain towards the margin, but these do not show anything which would afford a clue to its identity. It would seem that the specimen has been cut up for preservation, but if so the other pieces are not in Herb. Kew. It is not possible to determine anything from the specimen, and I never found anything to fit the description.

Marasmius proletarius B. & C.

This species from Cuba was described as white. The Ceylon painting, Thwaites No. 1170, shows a coloured pileus and represents young specimens of *M. coniatus*. The name should be deleted from the Ceylon list.

Marasmius radians B. & Br., Fungi of Ceylon, no. 366.

Thwaites no. 103 was divided by Berkeley and Broome into two species, *Cantharellus inaequalis* B. & Br., Fungi of Ceylon, no. 347, and *Marasmius radians* B. & Br. There was no painting. The two appear to be the same, and a *Cantharellus*, but until fresh specimens have been collected, the question may remain open. Both grew on wood or sticks.

Marasmius sulciceps Berk., Decades of Fungi, no. 156.

Pileus broadly convex, depressed, ochraceous, thin (? through drying), about 2 cm. diameter, minutely scurfy, no special pellicle; stalk compressed, cartilaginous, minutely pruinose, white, strigose at the base, with tawny mycelium spreading over the substratum, about 2 cm. long, stout when fresh, 1 mm. broad in the dry specimen; gills ventricose, adnate, moderately crowded; spores yellow brown, oval or obliquely oval, apiculate, 6 × 4 μ. On rotten wood.

This species has not been identified again. It was sent by Gardner, with a figure which shows a stout white stalk and a depressed ochraceous pileus. The radial grooves noted by Berkeley are probably the result of drying. It appears to be a *Naucoria*.

Marasmius Wynniae var. *auroricolor* B. & Br., Fungi of Ceylon, no. 353.

This is a *Mycena*, which may be known as *Mycena auroricolor* Petch, nom.nov.

Solitary or caespitose; pileus up to 2·5 cm. diameter, broadly convex, then almost plane, slightly depressed in the centre, margin striate when moist, pale lilac, rose in the centre, becoming entirely rose pink, or when young everywhere rose pink with a purplish tinge, pallid when old, sprinkled with minute glistening particles when dry; flesh thin, white in the centre; gills paler than the pileus, adnate or adnato-decurrent, narrow, 3 mm. broad, attenuated outwards, lower edge straight, interstices strongly veined; stalk up to 5 cm. long, 2·5 mm. diameter, equal, coloured like the pileus, hollow, smooth, shining, brittle, sometimes ridged above with decurrent lines from the gills, slightly strigose at the base; spores white, oval, 6–8 × 4 μ. On the ground among vegetable refuse. Peradeniya, 1 July 1908; 1 August 1909; also no. 4142.

The specimen Thwaites no. 204 cited by Berkeley and Broome as 'the same thing' is *M. leucophaeus*.

LATIN DESCRIPTIONS OF NEW SPECIES

Marasmius purpureo-griseus Petch, sp.nov. Fasciculatus; pileo late convexo, margine pallidiori, minute radiatim sericeo, ca. 1·5 cm. diam., margine incurvato; carneo albo tenui; stipite ad 4 cm. alt., 1·5 mm. diam., brunneo-albo, nitenti, cartilagineo, farcto dein cavo, fistuloso; lamellis confertis, albis vel cremeis, latis, adnatis v. sinuato-adnatis, acie recta. Ad lignum emortuum, mycelio albo affixus. Henaratgoda, Ceylon.

Marasmius rufo-ochraceus Petch, sp.nov. Fasciculatus v. sparsus; pileo late convexo v. fere plano et undulato, ad 3·5 cm. diam., centro rufo-brunneo, ad marginem ochraceo v. pallido, sicco toto ochraceo, radiatim sulcato, glabro, tenui, cartilagineo; stipite ad 3 cm. alt., compresso, ad 3 mm. lat., cartilagineo, rufo-brunneo, supra pallidiori, glabro v. minute tomentoso, farcto dein cavo; lamellis subremotis, subventricosis, adnatis, pallidis, brunnescentibus, aetate interstitiis valde venosis; sporis albis, clavatis, 10–14 × 5–6 μ. Ad lignum emortuum. Hakgala, Ceylon.

Marasmius brunneostriatus Petch, sp.nov. Pileo campanulato dein fere plano, obtuse umbonato, ad 2 cm. diam., rufo, centro saturatiori, margine

pallidiori flavo-brunneo, radiatim sulcato, sulcis saturatioribus, minute rugoso, margine saepe repando; stipite ad 2 cm. alt., 1 mm. diam., apice incrassato, cartilagineo, rufo-brunneo, pruinoso v. minute tomentoso, basi tomentoso, e strato tenui albo oriundo; lamellis pallido-ochraceis, distantibus, crassiusculis, angustis, aequalibus, adnatis; sporis albis, clavatis v. ovalibus, 5–9 × 3–4 μ. Ad folia et ramulos dejectos. Hakgala, Ceylon.

Marasmius senescens Petch, sp.nov. Pileo 1·5 cm. diam., late convexo, centro depresso, interdum umbonato, plicato-sulcato, centro corrugato, sordide griseo margine pallidiori, tenui, centro leniter tomentoso, alibi sparsim fibrilloso et hinc illic consperso; stipite 1 cm. alt., 1·5 mm. diam., supra expanso, albo v. cinereo, fuscescenti, compresso, striato, minute longitudinaliter sericeo, cartilagineo; lamellis distantibus, adnatis v. adnato-decurrentibus, circum apicem stipitis connexis et separantibus, latis, leniter ventricosis, crassiusculis, furcatis, pallidis, brunnescentibus, interstitiis venosis; sporis albis, angusto-ovalibus, subpyriformibus, 7–10 × 3–4 μ. Ad terram. Peradeniya, Ceylon.

Marasmius stypinoides Petch, sp.nov. Pileo albo, centro cinereo, late convexo v. fere plano, ad 5 mm. diam., umbilicato, leniter radiatim sulcato, minute rugoso, margine crenato; stipite ad 8 mm. alt., 0·4 mm. diam., saepius curvato, primo albo dein nigrescenti, pulverulento, striato, apice incrassato; lamellis albis, confertis, subventricosis, acie serrato. Ad corticem emortuum. Hakgala, Ceylon.

Marasmius cineraceus Petch, sp.nov. Pileo infundibuliformi, margine decurvo, leniter radiatim sulcato, 9 mm. diam., cinereo, levi, pellicula hypharum intertextarum; stipite nigro, albo-pruinoso, cartilagineo, 1 cm. alt., 0·75 mm. diam., aequali, e disco glabro rotundato oriundo; lamellis subconfertis, albis, sigmoideis, latis, adnato-decurrentibus, acie serrato, interstitiis venosis. Ad folia emortua. Peradeniya, Ceylon.

Marasmius subconiatus Petch, sp.nov. Pileo ad 5 mm. diam., late convexo, plicato-sulcato, umbilicato, obscure rufo-brunneo, particulis hyalinis consperso, cellulis pelliculae spinis conicis confertis coronatis; stipite ad 6 mm. alt., crasso, 0·4 mm. diam., nigro-brunneo, glabro, cartilagineo; lamellis numerosis (14), distantibus, cremeis, latis, acie recta v. subventricosis, late adnatis. Ad bambusam emortuam. Peradeniya, Ceylon.

Marasmius lateritius Petch, sp.nov. Pileo convexo v. conico-convexo, plano v. obtuse umbonato, ad 6 mm. diam., pallide rubra v. lateritio, saepe maculis flavis; stipite ad 2·5 cm. alt., 1 mm. diam., primo albo dein flavo-brunneo, corneo, minute hispido, basi strigoso; lamellis albis dein flavis, non distantibus, postice rotundatis v. truncatis, liberis sed stipitem approximatis; sporis albis, clavatis, 10–13 × 3–4 μ. Ad terram inter folia emortua. Peradeniya, Ceylon.

Marasmius albocapitatus Petch, sp.nov. Pileo candido, hemisphaerico, ad 4 mm. diam., minute umbilicato, leniter sulcato; stipite 1·5 cm. alt., 0·2 mm. diam., nigrobrunneo, setaceo; lamellis albis, angustis, adscentibus, adnatis, acie obtuso; sporis albis, anguste ovalibus, 8–10 × 3 μ; pileo et stipite crines longos patentes flavo-brunneos, attenuatos, acutos, infra 4 μ diam., ad 0·35 mm. alt. ferentibus; stipite etiam crinibus minutis, irregularibus, saepius curvatis, ad 30 × 6 μ, vestitis; cystidiis acie lamellarum ampullaceis, ad 34 μ alt., infra 12 μ diam. Ad folia emortua. Hakgala, Ceylon.

Marasmius micraster Petch, sp.nov. Pileo 3 mm. diam., udo fere plano, sicco campanulato et radiatim plicato, nigrobrunneo v. flavobrunneo, in sulcis saturatiori, membranaceo, centro depresso et corrugato, cellulis pelliculae ca. 16 μ diam., externe valde verrucosis; stipite setaceo, nigrobrunneo, glabro, nitenti, ca. 1 cm. alt., 0·2 mm. diam.; lamellis paucis, albis v. cremeis, ventricosis, liberis; cystidiis ampullaceis, ad 16 μ alt., apice truncatis; sporis albis, ovalibus, uno fine acutis, 10–12 × 4–5 μ. Ad ramulos emortuos. Peradeniya, Ceylon.

Marasmius tubulatus Petch, sp.nov. Pileo cylindrico-campanulato, profunde umbilicato, plicato-sulcato, pruinoso, membranaceo, ad 8 mm. diam., pallido dein brunneo-cinereo, margine pallidiori, centro nigro; stipite ad 2 cm. alt., 0·3 mm. diam., nigro-brunneo, corneo, glabro, nitenti, longitudinaliter striato; lamellis distantibus, albis dein pallidis, acie brunneo-griseo, subtriangularibus, postice in tubulo junctis; sporis albis, ovalibus v. clavatis, 7–9 × 3–4 μ. Ad folia et ramulos emortuos. Peradeniya, Ceylon.

Marasmius griseo-violaceus Petch, sp.nov. Pileo campanulato, ad 1 cm. diam., umbilicato, plicato-sulcato, membranaceo, griseo-violaceo v. purpureo-brunneo v. centro fere nigro alibi nigro-griseo, udo minute nigro-rugoso; stipite ad 7 cm. alt., 0·5 mm. diam., supra attenuato, rigido, corneo, nigro, nitenti; lamellis albis, distantibus, arcuatis v. triangularibus, acie colorati, in collarium liberum postice conjunctis; sporis albis, ovalibus, inaequilateralibus, 8–11 × 6 μ. Mycelio nigro rhizomorphoideo, 0·25 mm. diam. Basi *Dendrocalami gigantei* et ad folia emortua. Peradeniya, Ceylon.

Marasmius rigidichorda Petch, sp.nov. Pileo hemisphaerico, umbilicato, margine anguste decurvo, interdum infundibuliformi, plicato-sulcato, coriaceo, centro fusco-brunneo, alibi rubro-brunneo, fibris et punctis brunneis radiato, 1 cm. diam.; stipite ad 1 cm. alt., 1 mm. diam., nigro-brunneo, cartilagineo, scabro; lamellis distantibus, angustis, arcuatis, adnatis, rubescentibus dein cremeis, crassiusculis; sporis albis, angusto-ovalibus, inaequilateralibus, 8–10 × 3–4 μ. Mycelio aerio rhizomorphoideo, nigro, rigido, 0·4–0·6 mm. diam. Ad frutices. Hapugastenne, Ceylon.

Marasmius ascendens Petch, sp.nov. Pileo cinereo v. purpureo-griseo, ad 1 cm. diam., convexo dein plano v. repando, interdum umbonato, membranaceo, tenaci, minute rugoso; stipite ad 1 cm. alt., 0·2 mm. diam., setaceo, v. compresso, 0·4 mm. lat., glabro, corneo, infra nigro, supra purpureo-brunneo; lamellis distantibus, adnatis v. leniter decurrentibus, angustissimis. Mycelio aerio rhizomorphoideo, nigro, glabro, angulato, compresso et torto. Ad truncos et ramos arborum vivorum. Hakgala, Ceylon.

Marasmius Hakgalensis Petch, sp.nov. Pileo hemisphaerico v. late campanulato, ad 4 mm. diam.; purpureo-griseo v. cinereo-brunneo, centro saturatiori, membranaceo, minute rugoso, margine leniter striato; stipite ad 3 cm. alt., 0·5 mm. diam., purpureo-brunneo v. nigro, aequali, corneo, setuloso; lamellis subdistantibus, adnatis, albis, v. pallidis acie albo. Mycelio rhizomorphoideo, terrestri, nigro, angulato, setuloso dein glabro. Ad folia et ramulos emortuos. Hakgala, Ceylon.

Var. *denudata* Petch. Stipite glabro; lamellis magis confertis, purpurascentibus.

Marasmius Paspali Petch, sp.nov. Pileo hemisphaerico, coeruleo-griseo, leniter sulcato, pruinoso, 2·5 mm. diam.; stipite excentrico, nigrescenti, fibrilloso, cartilagineo, 2 mm. alt., 0·2 mm. diam.; lamellis ochraceis, latiusculis, paucis, adnatis, interstitiis venosis. Ad vaginas *Paspali dilatati*. Peradeniya, Ceylon.

Marasmius rufo-aurantiacus Petch, sp.nov. Pileo primo galeato, dein expanso, late campanulato v. fere plano, orbiculari, ad 2 cm. diam., primo rubro-brunneo, dein brunneo-cervino, levi, tenui, membranaceo, majoribus sulcatis; stipite excentrico, albo v. brunnescenti, minute pulverulento v. tomentoso, basi tomentoso, 2–3 mm. alt., 0·3 mm. diam.; lamellis vivide aurantiacis dein cervinis, adnatis, arcuatis, latiusculis, acie crasso, interstitiis venosis, acervulis cystidiorum cylindrico-clavatorum massis rufis coronatorum maculatis; sporis albis, late clavatis, apice leniter curvatis, 8–12 × 4 μ. Ad corticem et ramulos emortuos. Hakgala, Ceylon.

Marasmius porphyreticus Petch, sp.nov. Pileo saepius galeato, dein fere plano, orbiculari, stipite excentrico, v. convexo, stipite fere centrali, purpureo-brunneo, rugoso, plicato-sulcato, ad 2 cm. diam.; stipite brevi, 1 mm. diam., supra incrassato, purpureo-brunneo, glabro; lamellis paucis, purpureo-brunneis, distantibus, adnatis, saepius furcatis, crassis; sporis albis angusto-ovalibus, 7–11 × 3–4 μ. Ad ramulos emortuos. Hakgala, Ceylon.

Marasmius alliarius Petch, sp.nov. Pileo orbiculari, ad 2 cm. lat., late convexo, membranaceo, levi, rufo-brunneo, margine sulcato; stipite excentrico, brevissimo, supra incrassato, longitudinaliter sericeo, albo; lamellis cremeis, majoribus adnatis, arcuatis, latis, interstitiis venosis; sporis albis, ovalibus, 7–10 × 5 μ. Contuso alliatus. Ad lignum et ramulos emortuos. Hakgala, Ceylon.

Marasmius purpureo-albus Petch, sp.nov. Pileo saepius orbiculari, ad 3 cm. diam., convexo, repando, stipite excentrico, interdum stipite centrali, late convexo, primo pallide purpureo, dein albo v. pallide ochraceo, postice depresso v. umbilicato, vage plicato, glabro; stipite albo, crasso, ad 1 cm. alt., medio 2 mm. diam., aequali v. supra incrassato, basi annulari-tomentoso; lamellis distantibus, ventricosis, majoribus sinuato-adnatis, albis v. pallidis; sporis albis, angusto-ovalibus, inaequilateralibus, 10–14 × 5–6 μ. Ad caulem marcidum *Musae* (Banana), et ad corticem vivae *Lagerstroemiae*. Peradeniya, Ceylon.

Marasmius Amomi Petch, sp.nov. Pileo primo convexo, purpureo-brunneo, spongioso-tomentoso, dein late convexo v. plano v. vage repando, ad 5 cm. diam., vage radiatim plicato, postice purpureo-brunneo, alibi pallide ochraceo, innatis radiantibus fasciculis purpureo-brunnearum hypharum sparsim induto; stipite excentrico, albo, glabro, supra incrassato, ad 1 cm. alt., 1·5 mm. diam., basi aurantiaco-tomentoso; lamellis albis v. purpurascentibus, distantibus, adnatis, furcatis, interstitiis venosis. Ad caules emortuos *Amomi*. Hakgala, Ceylon.

Marasmius Calami Petch, sp.nov. Pileo ad 2 cm. diam., late convexo, membranaceo, super lamellis valde sulcato, inter vage bullato, viridi-albo,

margine udo fimbriato crinibus clavatis infra 3 μ diam., supra 4–8 μ; stipite excentrico, curvato, connexo, ad 1 cm. alt., 1 mm. diam., aequali, apice incrassato, sericeo-striato; lamellis albis, crassiusculis, distantibus, adnato-decurrentibus, saepe furcatis, venis validis connexis; sporis albis, clavatis, 13–16 × 4–5 μ. Ad caulem emortuum *Calami*. Peradeniya, Ceylon.

EXPLANATION OF PLATES

(All figures natural size, unless otherwise stated; numbers in brackets are those of the specimens from which the figures were drawn.)

PLATE II

Fig. 1. *M. nephelodes*, and section.
Fig. 2. *M. rivulosus*, full-grown, young, and section (4380).
Fig. 3. *M. Caryotae*, and section.
Fig. 4. *M. hirtellus*, and surface view of pileus (4294).
Fig. 5. *M. pallido-rubens*, and section (4382).
Fig. 6. *M. crispatus*, and under-surface (4239).
Fig. 7. *M. brunneostriatus*, and section (4116).
Fig. 8. *M. calvus*, and section (4115).
Fig. 9. *M. proximus* (4152).
Fig. 10. *M. corticigena*, full grown, section, and young (4370).
Fig. 11. *M. porphyreticus*, upper and under-surface (4126).
Fig. 12. *M. alliarius*, upper and under-surface (4131).
Fig. 13. *M. rufo-aurantiacus*, under and upper surface (4130).
Fig. 14. *M. Campanella*, small specimen (4124).

PLATE III

Fig. 1. *M. congregatus*, and section.
Fig. 2. *M. leucophaeus*, large example (4215).
Fig. 3. *M. purpureo-albus*, upper and under-surface, from banana (5303).
Fig. 4. *M. pulcher*, × 2 (5269).
Fig. 5. *M. subcinereus*, arboreal form, under-surface, × 2 (6542).
Fig. 6. *M. Campanella*, ochraceous form (4748).
Fig. 7. *M. purpureo-griseus* (5736).
Fig. 8. *M. equicrinis*, and under-surface.
Fig. 9. *M. tubulatus* (4243).
Fig. 10. *M. purpureo-albus*, central (6557) and lateral (6541) stalked, from *Lagerstroemia*.
Fig. 11. *M. Paspali*, × 2 (6531).
Fig. 12. *M. nivosus* (6552).
Fig. 13. *M. hirtellus* var., and upper surface, × 2 (5807).
Fig. 14. *M. subcinereus* (6627).
Fig. 15. *M. multijugus*, and section (6256).

PLATE IV

Fig. 1. *M. coniatus* (4221).
Fig. 2. *M. Leveillianus*, and section (4625).
Fig. 3. *M. florideus*, large example (4207), and section of small example (4281).
Fig. 4. *M. haematocephalus* (4102).
Fig. 5. *M. lateritius*, and section (4375).
Fig. 6. *M. fulviceps* (4077).
Fig. 7. *M. gordipes*, and under-surface (4235).
Fig. 8. *M. semipellucidus*, and section (4305).
Fig. 9. *M. Thwaitesii*, and under-surface (4353).
Fig. 10. *M. hypochroides*, large example (4244).
Fig. 11. *M. hypochroides*, small example and section.
Fig. 12. *M. Hakgalensis* (right) (4133), and var. *denudata* (left) (4125).
Fig. 13. *M. helvolus* var. *brunneolus* (4204).
Fig. 14. *M. griseoviolaceus*, and under-surface (4238).
Fig. 15. *M. albocapitatus*, and under-surface (4127).
Fig. 16. *M. actinophorus* (4134).
Fig. 17. *M. stypinus*, young (left), full grown (middle) and section (right) of young example (4248).
Fig. 18. *M. Campanella*, under-surface (4122).

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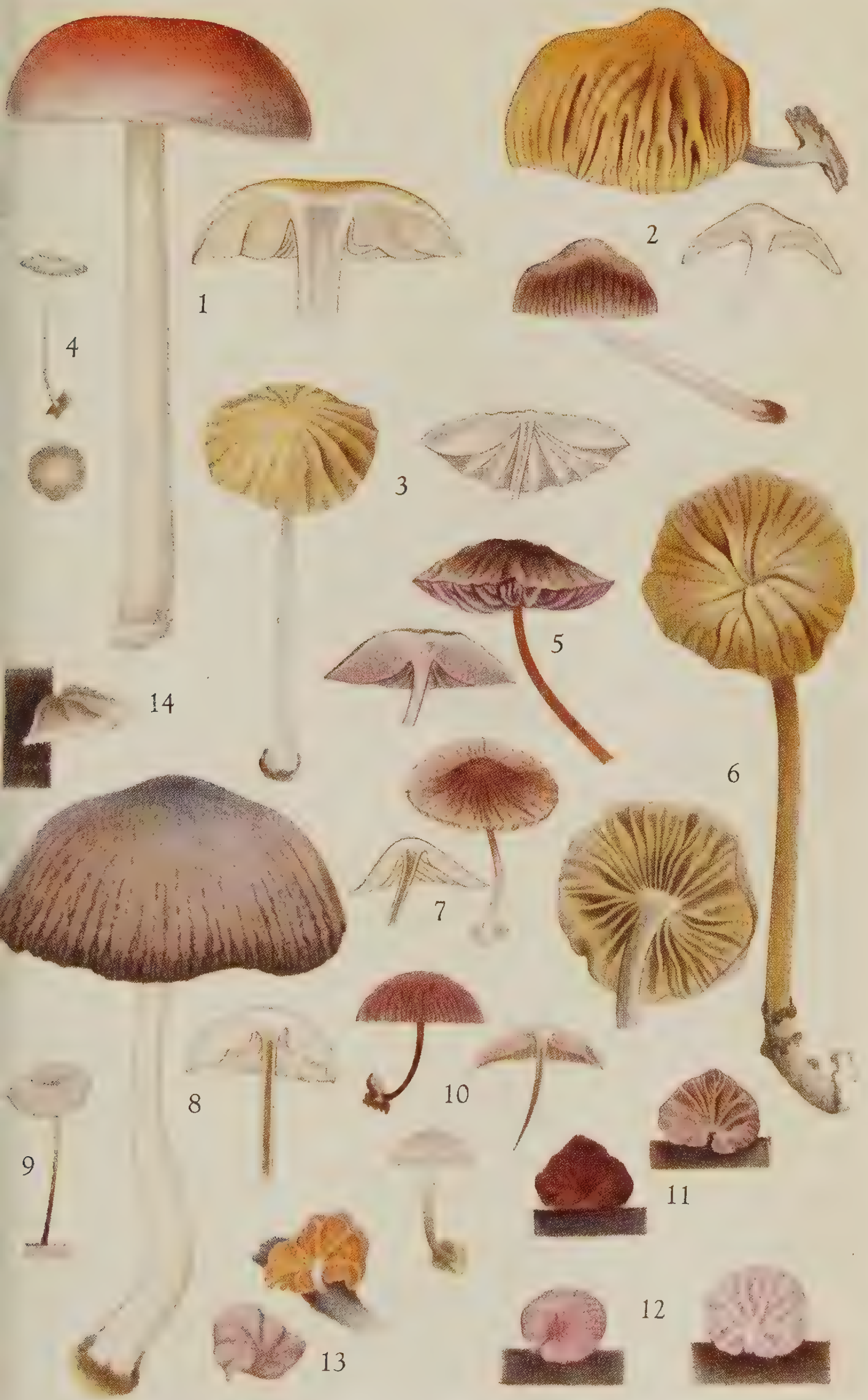
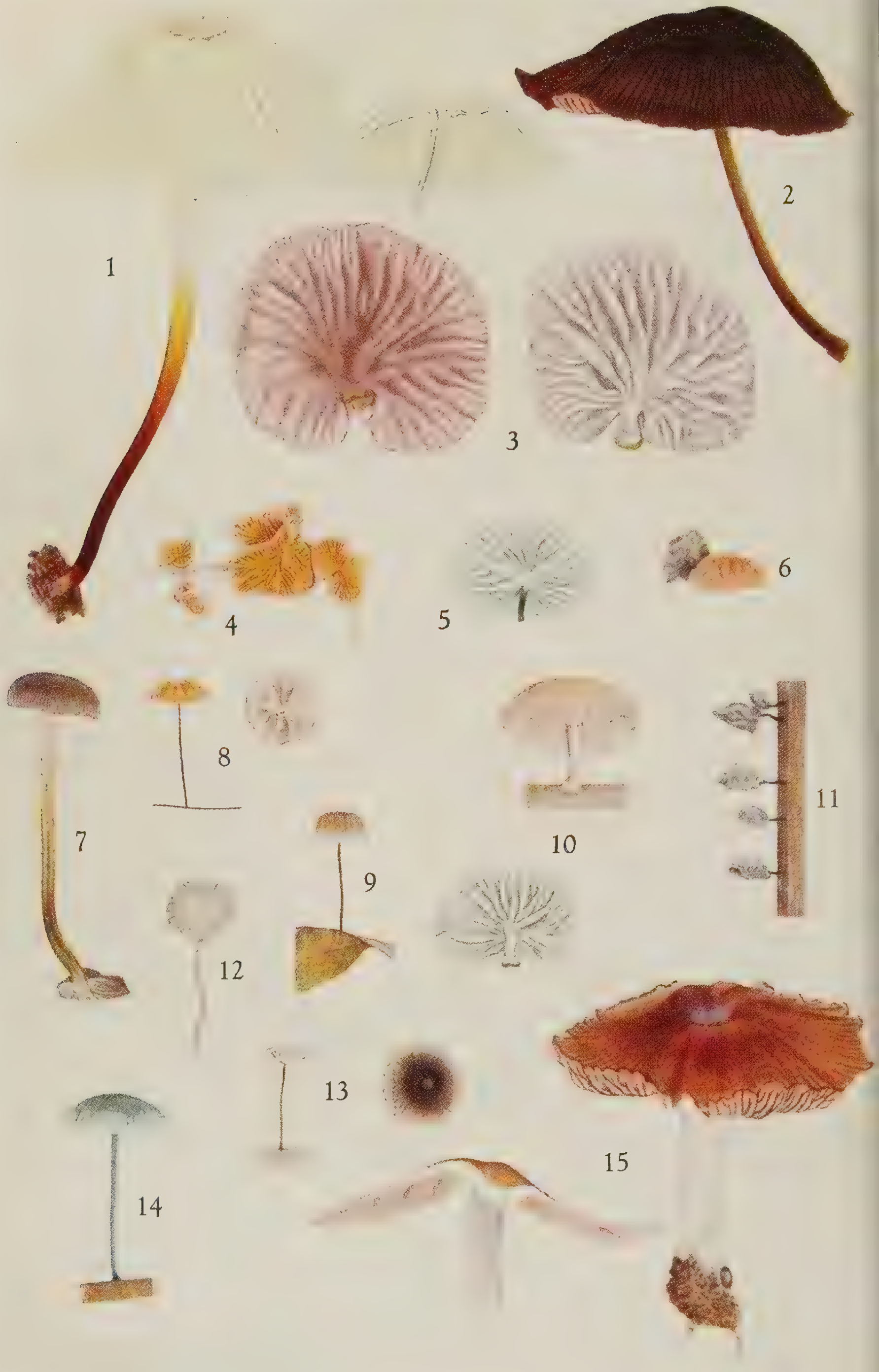


A. George Alwis del.

Ceylon *Marasmii*



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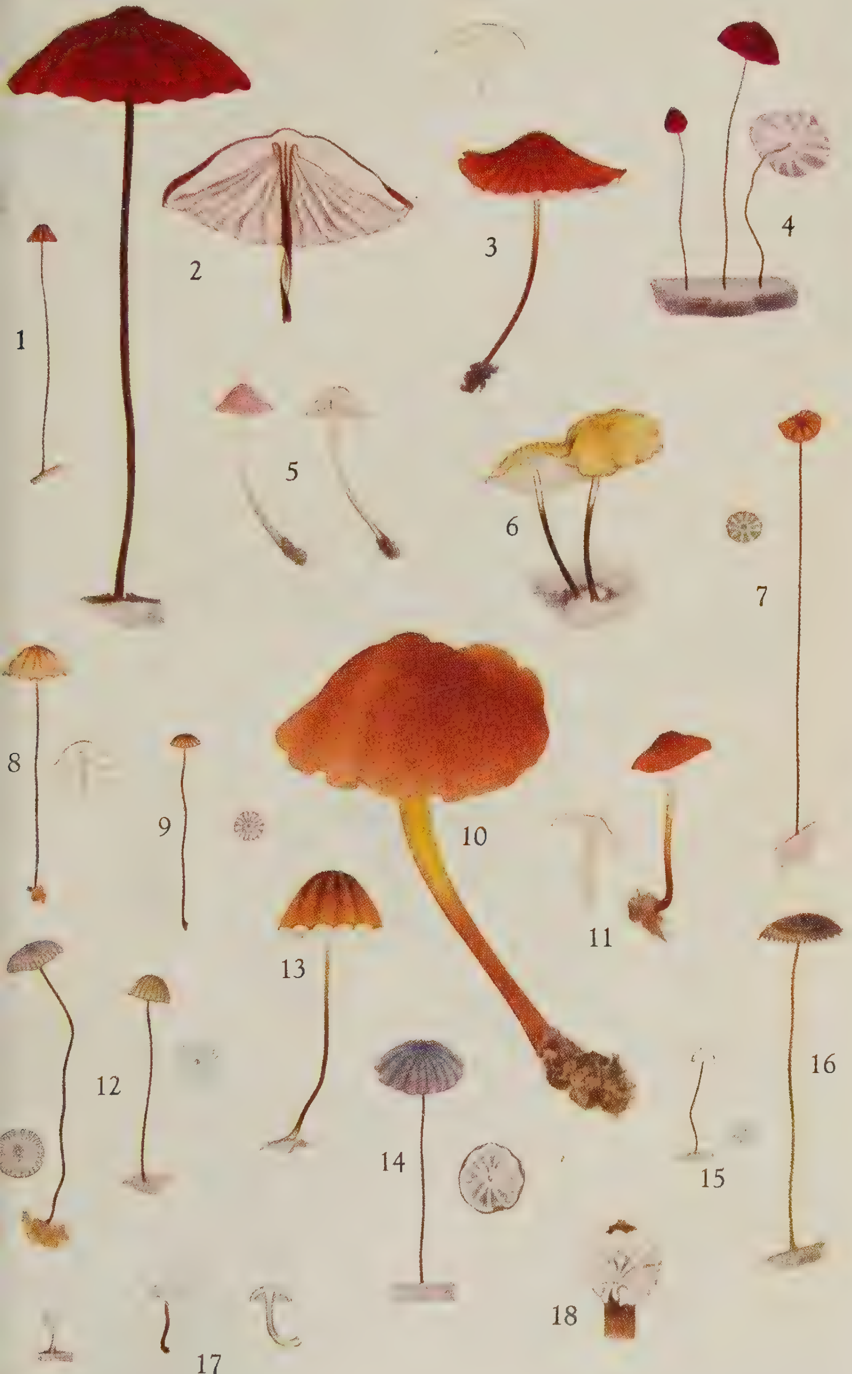
Ceylon *Marasmii*



A. George Alwis del.

Ceylon *Marasmii*

THE DEPENDENCE ON THE WEATHER OF THE DATES OF OUTBREAK OF POTATO BLIGHT EPIDEMICS

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THE DUTCH RULES FOR FORECASTING BLIGHT EPIDEMICS

Many observations have been made on the relation between weather conditions and the dates of outbreak of potato blight epidemics. Very little of practical value was discovered until van Everdingen (1926), from an examination of Dutch weather data, established four conditions which must be satisfied before outbreaks of blight occur. The value of this work was that, for the first time, it enabled forecasts to be made of the probable occurrence of blight and, therefore, of the date when spraying to control the disease should be begun.

Van Everdingen's conditions (which will be referred to here as the Dutch rules) were as follows:

(1) Dew occurring for at least four hours during the night.
(2) Minimum night temperature not less than 10° C. (50° F.).
(3) Mean cloudiness of the day following the dew not less than 0·8.
(4) Rainfall on the day following the dew of at least 0·1 mm.

If any day occurred on which all these conditions were obeyed, blight usually made its appearance within a fortnight.

These rules have been applied successfully in Holland and a spray warning service organized there (van Poeteren, 1928, 1931; van Everdingen 1935). They have also been tested in other countries. Wiltshire (1931) applied them to data from a number of places in England and Wales for the years 1921–7. Although the rules were often obeyed, the results on the whole were less satisfactory than in Holland. Better results were obtained when slight deviations were permitted in the application of the rules (e.g. three hours' dew formation instead of four, or 49° F. instead of 50° F.), and such a procedure seemed reasonable because the conditions governing biological phenomena can rarely be expressed with rigid arithmetical precision. This has the practical drawback, however, that it becomes much more difficult to apply a spray warning service with confidence because there is a large increase in the number of almost favourable days which are not followed by blight within a fortnight. I have applied the Dutch rules to the weather data at Newton Abbot, Devon (Beaumont, 1929–32) and, as will be seen, have come to the same conclusion as Wiltshire. The Dutch rules were satisfactorily applied by Dufrenoy (1933) at Bordeaux in 1932 and by Naoumova (1935) in the neighbourhood of Leningrad in 1933 and 1934.

It may be useful at this stage to discuss the phytopathological significance of the rules. Thanks to the work of Melhus (1915), Crosier (1934) and others, we have a great deal of information about the biology of the blight fungus which is directly relevant to the rules. Blight appears in the crop as a result of planting one or more infected tubers, in the aerial shoots from which the blight fungus develops as soon as conditions are favourable. There are two stages preceding the first visible appearance of blight in what may be called primary infection foci, namely, growth of mycelium in the shoot and formation of conidia. The first stage is governed only by temperature, which is covered by the second of van Everdingen's rules, and which will have a cumulative effect during the growth of the plant. Crosier (1934) has shown that the fungus grows most rapidly at 18–21° C. (64–69° F.) and very slowly below 9° C. (48° F.) or above 24° C. (75° F.). The formation of conidia is governed both by temperature and humidity: the optimum temperature is 18–22° C. (64–72° F.).

The other three rules of van Everdingen relate to the dissemination of blight rather than to the formation of primary infection foci. Naturally, if conditions were quite unfavourable to the spread of blight, the disease would not be reported, as it is unlikely that during ordinary field observation the primary infections would be noticed. It is well known that infection by *Phytophthora infestans* takes place most readily when zoospores are formed, and this occurs only when there is a film of moisture on the leaf. The longer the film of moisture persists the greater is the chance of infection. Dew formation (rule 1) followed by cloudy skies (rule 3) and a certain amount of rain (rule 4) mean that the moisture will last a considerable time and the rules therefore allow for the conditions under which blight will spread sufficiently to form a noticeable outbreak.

Application of the Dutch rules to South Devon

Difficulties arise when using the Dutch rules owing to the nature of the meteorological data to be consulted. Dew formation cannot be directly observed, but the period may be estimated by comparing the continuous temperature record with the dew points recorded at 21 hr. (the night before) and at 9 hr. in the morning. It is by no means certain how closely the period obtained in this way corresponds with the actual period of dew formation on potato leaves. Dew does not readily form on potato leaves; much less readily, for example, than on grass. At most stations continuous temperature records are not available and in Table 1 dew formation has been regarded as occurring if the minimum temperature (read at 9 a.m.) was more than 2° F. below the dew point (also read at 9 a.m.).

The meteorological data for the amount of cloud usually refer to one observation only (9 a.m.) and in no case to more than three, and in 1931 they ceased to be recorded at most English stations. The sunshine record would seem to give a better idea of the general cloudiness during the day and in Table 1 a maximum of five hours' sunshine has been allowed.

The rainfall condition is most variable in its application. A combination of a little rain and little sunshine generally means persistent moisture on the leaves. But rain in the morning is often succeeded by wind and intermittent sunshine during the afternoon, and potato leaves dry up very readily under these conditions. It cannot be expected that the variety of eather conditions can adequately be summed up in a simple rule, and hese limitations must be accepted.

Another difficulty is that the nearest meteorological station for which ecorded data are available may be some distance from the potato field. his was unavoidably so in Wiltshire's observations, but in the Newton bbot results given below the meteorological instruments were either in or ery close to the potato field.

In arriving at Table 1 the Dutch rules have therefore been modified as llows:

(1) Minimum temperature not less than 2° F. below the dew point.
(2) Minimum temperature not less than 50° F.
(3) Sunshine of the following day not more than five hours.
(4) Rainfall of the following day at least 0·1 mm.

Table 1. *South Devon*

1 Year	2	3	4	5	6	7	8
1929	11 July	4	1	39	1	1	8
1930	2 Aug.	3	2	43	3	0	52
1931	10 June	3	2	3	3	3	4
1932	15 July	4	1	42	2	0	41
1933	29 July	7	2	58	2	1	57
1934	16 Aug.	9	5	57	0	0	—
1935	26 Aug.	10	3	81	3	1	66
1936	25 July	8	2	34	3	1	22
1937	20 July	8	5	28	0	0	—
1938	8 Aug.	8	1	51	1	1	9
1939	24 July	7	3	32	1	0	18
		71	27	468	19	8	277
				Mean 42·5			Mean 31

Table 1*a*. (*The same excluding* 1931 *and excluding May and June*)

1 Year	2	3	4	5	6	7	8
1929	—	1	1	9	1	1	8
1930	—	2	2	6	1	0	18
1932	—	1	1	2	0	0	—
1933	—	4	2	21	1	1	11
1934	—	7	4	35	0	0	—
1935	—	4	3	55	1	1	1
1936	—	6	3	24	3	1	22
1937	—	6	5	17	0	0	—
1938	—	4	1	32	1	1	9
1939	—	5	3	20	1	0	18
		40	25	221	9	5	87
				Mean 22			Mean 12

Column 2. Date of outbreak of blight.
3. No. of critical periods preceding outbreak (Dutch rules).
4. No. of critical periods in the fifteen days preceding outbreak (Dutch rules).
5. No. of days between first critical period and outbreak, excluding May (Dutch rules).
6, 7 and 8. As 3, 4 and 5 respectively but determined according to the temperature-humidity rule.

The data obtained from the weather records (not published here owing lack of space) are summarized in Table 1. Examination of columns 3, 4 and 5, obtained by the application of the Dutch rules to the weather data, shows that the outbreak of blight was preceded within fifteen days by one or more critical days in every year from 1929 to 1939. If there had been only one such day before the blight outbreak an accurate forecast of the latter could have been made. With two or three critical days within the fifteen-day period, it would still be possible to give a useful and closely approximate forecast. But in every season critical days occurred which were more than fifteen days before the outbreak and in every year except 1931 the first critical day recorded was, in fact, a much longer period ahead of the blight outbreak. There were indeed forty-four such days out of a total of seventy-one, or 62 %. Even if we consider a twenty-one-day interval as of practical value (i.e. forecasts of blight would take the form of possible outbreaks any time up to three weeks from the date of forecast) the percentage of premature forecasts would still be as much as 56 %.

A possible explanation of the number of premature forecasts is that the chances of blight appearing in epidemic form vary in the different months according to the growth of the crop. Obviously the chances of blight appearing before the shoots are through the ground are nil, whatever the weather, but as growth proceeds and leaves and shoots become more numerous the chances of blight will become greater. In other words, a favourable period in July is more likely to be followed by blight than one in June, while in May the chances are still less. Critical days are rare in May because the temperature is generally too low (only one day in each of seven seasons out of the eleven). In the seasons under investigation blight appeared in June only once (1931). In Table 1 *a* critical days earlier than July are disregarded. We now find that all the critical days are followed by blight within fifteen days in three seasons out of ten, and within twenty-one days in six seasons. The percentages of premature forecasts are reduced to 37 and 15 % respectively. This is a big improvement. Apart from 1931, therefore, good results were obtained when considering only the July and August data. It is, however, important to be able to forecast when blight may come exceptionally early, as it did in 1931, because spraying earlier than usual would have been essential in that year. The application of the Dutch rules does not indicate why 1931 was so exceptional.

THE TEMPERATURE-HUMIDITY RULE

The Dutch rules do not take into account the relative humidity of the atmosphere, perhaps because adequate data were not available. The importance of high humidity in relation to the formation of conidia is well known and should be considered (Beaumont, 1930). This was done, and it was found that humidity and temperature alone were sufficient to give a practically useful indication of the likelihood of blight. When the data are available the application of a 'temperature-humidity rule' is much simpler than the use of the Dutch rules.

When this rule was first adopted relative humidity records were being made at 9, 15 and 21 hr. and these enabled a fairly reliable picture to be obtained of the degree of humidity throughout the day. Unfortunately nly the 9 hr. reading was made from 1936 onwards, and as relative umidity usually diminishes during the afternoon such restricted data are f no value for our present purpose. This restricts the application of new ules to the few stations that possess continuous recording instruments or ake sufficient hygrometric readings during the day. It is true that the bsolute humidity of the air varies little during the day, and the relative umidity, which depends on temperature, can be calculated if the latter is nown. Nevertheless, it would be a great help if direct readings of relative umidity at 15 hr. could be re-started. The Meteorological Office (1928) ecognized that 'important variations in relative humidity occur during he hours of daylight between 7 and 21 hr. and are not adequately isclosed by observations at 7, 8, 9, 18 or 21 hr. An observation in the arly afternoon is required to indicate the extent of the fluctuations'. The 3 hr. reading given in the Daily Weather Map goes some way to supply his need.

During a period of high humidity there is an absence of wind, and the rop in temperature that always occurs at night is often sufficient to induce ew formation, while the film produced does not dry up readily owing to he dampness of the air. This is especially likely when the high humidity ersists for forty-eight hours. In effect, therefore, the humidity conditions omprise in one rule the Dutch rules 1, 3 and 4.

The new conditions proposed may be stated as

(1) Minimum temperature not less than 50° F.

(2) Relative humidity not below 75 % for at least two days.

Table 1 gives the number of critical periods preceding the outbreak of light in each year, a critical period being defined as one in which the equired temperature and humidity were both observed for two or more onsecutive days. For comparison with the Dutch rules the total number of eriods and the number of periods up to fifteen days before the outbreak re both given, as well as the number of days after the first critical period hen blight was noted. In 1929 and 1930 only three humidity readings, at a.m., 3 p.m. and 9 p.m., were available, but in subsequent seasons the ata were taken from a recording hygrometer kept in a screen placed mong the potatoes.

Columns 6, 7 and 8 show that blight was preceded by one or more ritical periods, not in every season, as with the Dutch rules, but in nine easons out of eleven. On the basis of the temperature-humidity rule, herefore, no spray forecast would have been issued in 1934 and 1937. ctually the epidemics were very slight in those years, so that the practical isadvantages of the failure to forecast are not great, a point that will be iscussed further below. The temperature-humidity rule has the advantage ver the Dutch rules in that in any given season the number of critical eriods is smaller, so that a forecast can be made with more certainty compare column 6 with column 3). The table shows that in 1929 and 1938 here was only one critical period, and in 1931 all the three critical periods ere within the fifteen days' period. In these three seasons, therefore, nequivocal forecasts could have been issued. In 1939 the single critical eriod was within the twenty-one-day interval. In four seasons out of eleven, therefore, blight followed all the critical periods within twenty-one days. This was not so with the Dutch rules in any single season, unless dates before July were excluded. If we consider July in relation to the temperature-humidity rule, we find that blight followed within fifteen days in four seasons out of ten and within twenty-one days in six seasons out of ten (Table 1 *a*). This is very similar to the results from the Dutch rules. Examination of column 8 shows, however, that fairly good forecasts could have been made every year except in 1932, 1934 and 1937 when there would have been no forecasts.

A comparison of the general results of the application of the two sets of rules indicates that the Dutch rules give rather too many critical periods and the temperature-humidity rules too few. Might not a slight modification of the temperature-humidity rules lead to their wider application? It is possible, for example, that a period of one or two hours of slightly lower relative humidity than 75 % would not check the effect of the two-day period in bringing about the production of conidia. When the weather data available for 1931–9 are re-examined to allow for this, the total number of critical periods is increased from nineteen to twenty-eight, and accurate forecasts can be made of the slight outbreaks in 1934 and 1937 not previously forecasted. The percentage of critical periods within the fifteen-day period, out of the total observed before the outbreak, is, however, only slightly greater in this wider application, i.e. fourteen out of twenty-eight (50 %) instead of eight out of nineteen (42 %), and for the twenty-one-day period sixteen out of twenty-eight (57 %) instead of eleven out of nineteen (58 %). The advantage of a slightly wider interpretation of the humidity rule is therefore negligible.

The important exception of the June outbreak of 1931 may now be considered. Humidity and temperature were both favourable on 28, 30 and 31 May, and on 5, 6, 8, 9, 10 and 11 June, i.e. on nine days out of fifteen. In no other year were there more than four such days in this period. Again, the number of days in May 1931 with the requisite temperature and humidity was six: in other years the number ranged from none to three. If we examine the Dutch rules for the same period there is no such outstanding indication: the number of favourable days before 10 June, the date of outbreak, was only three. There were also three days before this date in 1933 and two in 1929, 1932 and 1935. Also in May 1931 and in six other seasons there was only one favourable day according to the Dutch rules.

It may thus be concluded that from 1929 to 1939 both the Dutch rules and the temperature-humidity rule gave useful indications of blight in South Devon, and that the temperature-humidity rule was slightly more precise and simpler to apply.

APPLICATION OF THE TEMPERATURE-HUMIDITY RULE TO OTHER DISTRICTS

West Cornwall. Conditions in West Cornwall are particularly interesting because the potato crop consists of early varieties put in the ground very early and often dug before blight has appeared in other parts of England.

Limited crop rotation and close planting favour the progress of the disease. It might therefore be expected that the conditions preceding a blight outbreak might correspond less closely to the rules than in South Devon. Investigation has shown that this is not so, but that, as will be seen in Table 2, the rules apply to West Cornwall equally satisfactorily.

Table 2. *Potato blight in West Cornwall* 1931–9

Year	Date of outbreak	Number of critical days preceding outbreak of blight		Remarks
		Up to 15 days	Over 15 days	
1931	12 May	1	0	
1932	30 May	2	0	
1933	7 May	1	1	
1934	16 May	0	0	Very local
1935	—	1	0	
1936	—	0	0	
1937	20 May	1	0	
1938	—	2	0	Crop lifted early
1939	—	0	0	

The data in the table for 1931 and 1932 were prepared from records taken at Gulval, which is in the centre of the early potato area. Early afternoon readings of humidity were not taken after 1932 and the data for the remaining years were obtained from the Daily Weather Map, which gives the minimum temperature at Penzance and the 13 hr. relative humidity at the Lizard. These are less satisfactory, as the minimum temperature is often slightly lower at Penzance than at Gulval, and at the Lizard there is generally a higher humidity. To correct this a minimum of 80 % humidity was taken for the table.

The records show that blight followed the first critical periods within fifteen days in three seasons out of the four in which blight occurred in the district. In the fourth season (1933) blight followed thirty-five days after the first critical period and two days after the second. There was no critical period and no blight in 1936 and 1939; and in 1934, 1935 and 1938 the first critical period came too late, most of the crop having been lifted before blight made its appearance.

In 1931 the critical period coincided with the outbreak of blight, which was probably governed by the weather of 28 and 29 April and 5, 6 and 7 May, when the slightly lower minimum temperatures that prevailed on those dates, coupled with the high humidity, were still sufficient to bring about blight. This was also noticed for earlier seasons by Wiltshire (1931).

France. The temperature-humidity rule was tested in France in 1938 by Limasset (1939). Observations at Versailles showed that there was no favourable period until September, the only days following the rules being 26 July, 23 August, and 20, 25, 26 and 27 September. Spread of blight began early in August but was slight; and as was found for Newton Abbot in 1934 and 1937, the method was not successful in forecasting the start of a slight epidemic. As pointed out by Limasset, under such conditions the microclimate determines the extent of the disease in any given locality and cannot be represented adequately by a single set of observations. At the end of September, when the epidemic became more extensive, the rules could be correctly applied.

Other observers in the Hautes Pyrénées in 1938 found that the outbreak of blight (20 August) followed a favourable period from 15–18 August in which the rules were obeyed (Ministère d'Agriculture, 1939, p. 114).

SUMMARY

A detailed study has been made of the meteorological data recorded at Seale-Hayne Agricultural College, Devon, during the eleven years 1929–39, in relation to outbreaks of potato blight, and it has been shown that the Dutch rules governing the date of the blight outbreak also apply in South-west England. Their practical value as a basis for forecasts is lessened by the fact that favourable days occur which are not soon followed by blight, so that the application of the rules in Devon in every season except 1931 indicated blight earlier than it appeared. During the period in question blight appeared in June only in 1931. Excluding this year and considering only the July and August weather data it was found that blight followed the first critical date within fifteen days in three seasons out of ten and within twenty-two days in six seasons out of ten. The rules provided no explanation of the early outbreak of 1931.

It is difficult to obtain the necessary data for the application of the Dutch rules, and a simpler modification has been devised and called the 'temperature-humidity rule'. The conditions are (1) minimum temperature not less than 50° F. and (2) relative humidity over 75 % for two consecutive days. This rule has been found to give good results in Devon and West Cornwall most seasons. Blight followed the first critical dates within fifteen days in three seasons out of eleven and within twenty-two days in five seasons out of eleven. The data also throw light on the exceptionally early outbreak of 1931. Excluding this year and considering only the July and August weather records it was found that blight followed the first critical period within fifteen days in four seasons out of ten and within twenty-two days in seven seasons out of ten. In the remaining three seasons there was no critical period. This failure to indicate blight was not a disadvantage from the practical point of view because the blight epidemic was so slight in those seasons.

On the whole the temperature-humidity rule is regarded as slightly more useful for blight forecasting than the Dutch rules.

REFERENCES

Beaumont, A. (1929–32). *Rep. Dep. Pl. Path. Seale-Hayne agric. Coll.*

Beaumont, A. (1930). *Rep. Dep. Pl. Path. Seale-Hayne agric. Coll.* p. 22.

Beaumont, A. (1940). Potato blight and the weather. *Trans. Brit. mycol. Soc.* xxiv, 266.

Crosier, W. (1934). Studies in the biology of *Phytophthora infestans* (Mont.) de Bary. *Mem. Cornell agric. Exp. Sta.* no. 155.

Dufrénoy, J. (1933). *In* Rapports sommaires sur les travaux accomplis dans les laboratoires en 1932. *Ann. Épiphyt.* xix, 1–46. Abstract in *Rev. appl. Myc.* xiii, 76.

Limasset, P. (1939). Recherches sur le *Phytophthora infestans* (Mont.) de Bary. *Ann. Épiphyt.* n.s. v, 21–39.

Melhus, I. E. (1915). Germination and infection with the fungus of the late blight of potato. *Bull. Wisconsin agric. Exp. Sta.* no. 37.

Meteorological Office (1928). *The Book of Normals of Meteorological Elements of the British Isles.* Section VI. *Normals of Relative Humidity.*

Ministère d'Agriculture (1939). *Comptes rendus sommaires des Travaux des Stations et Laboratoires de Recherches Agronomiques en* 1938.

Naoumova, N. A. (1935). [On forecasting the appearance of *Phytophthora infestans* on the potato.] *Pl. Prot. Leningrad*, iii, 51–4. Abstract in *Rev. appl. Myc.* xv, 522.

Van Everdingen, E. (1926). Het verband tusschen de weergesteldheid en de aardappelziekte (*Phytophthora infestans*). *Tijdschr. PlZiekt.* xxxii, 129. Abstract in *Rev. appl. Myc.* v, 627.

Van Everdingen, E. (1935). Het verband tusschen de weergesteldheid en de aardappelziekte (tweede mededeeling). *Tijdschr. PlZiekt.* xli, 125–33. Abstract in *Rev. appl. Myc.* xiv, 715.

Van Poeteren, N. (1928). Een waarschuwingsdienst voor het optreden van de aardappelziekte. *Versl. PlZiekt. Dienst Wageningen*, no. 53. Abstract in *Rev. appl. Myc.* xi, 95.

Van Poeteren, N. (1931). Verslag over de werkzaamheden van den Plantenziektenkundigen Dienst in het jaar 1930. *Versl. PlZiekt. Dienst Wageningen*, no. 64. Abstract in *Rev. appl. Myc.* xi, 95.

Wiltshire, S. P. (1931). The correlation of weather conditions with outbreaks of potato blight. *Quart. J. R. met. Soc.* lvii, 304–16.

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THE ECOLOGY OF *ERYSIPHE GRAMINIS* DC.

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(With Plate V and 7 Text-figures)

Corn mildew, caused by the fungus *Erysiphe graminis* DC., can assume serious economic proportions in the west of Scotland, when cereal crops are grown for green fodder. It causes some damage on grain crops, but probably develops too late in the growing period to curtail the production of grain very seriously.

Studies on the ecology and biology of *E. graminis* in various countries have given somewhat discordant results, and it is probable that different strains are involved on the same crop in different regions. There are, of course, strains upon various grasses and cereals which will not inter-infect. The strain chiefly used in the present experiments appears to attack only oats; it will not infect wheat. These investigations were planned to acquire information as to the factors responsible for the severity of attack by *E. graminis* in the west of Scotland. They evaluate some aspects of the major factors of climate, soil nutrition and composition of the host plant. Literature on *E. graminis* has been adequately reviewed by Cherewick (1944).

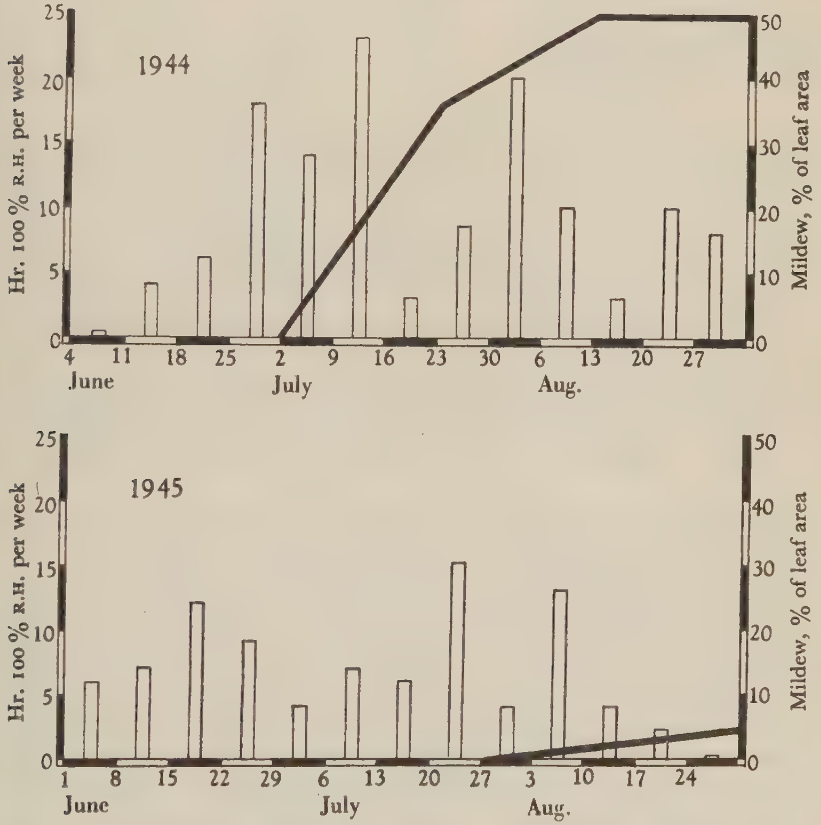
The effect of climate

Preliminary correlations of climate and the severity of corn mildew, suggest that the fungus owes more to increase in relative humidity (R.H.) than to increase in summer temperature, in the severity of its attack (Table 1). Records showed, moreover, that the R.H. associated with the heaviest infection of mildew (greenhouse, Table 1), reached 100% on six days out of the forty-two covered by the preliminary inquiry. The R.H. of the other two sites never attained saturation at 9 a.m., when the readings were taken.

Table 1. *General climatic relations of* Erysiphe graminis *on oats*

Site	Mildew % of leaf area	During development of disease Av. temp. ° F.	Av. % R.H.
Plant Pathology fields, Auchincruive	20	59	79
Oat trials, Auchincruive	25	69	77
Greenhouse, Plant Pathology Dep.	65	59	88

It is suspected that the disease becomes severe when atmospheric humidity reaches saturation for appreciable periods. That this idea has some foundation in fact is shown by Text-fig. 1, which integrates the number of hours of 100% R.H. per week with the percentage of leaf area overed by the fungus. The humidity was computed from a continuous ygrograph record; the infected leaf area was estimated by comparison ith 'standard area' diagrams (see Appendix). The year 1944, with a large umber of hours of 100 % R.H. per week allowed a development of the ngus to cover 50 % of the leaf area; 1945, with fewer hours of saturation er week, had a much lower infection of about 5 %. These figures refer to e general level of mildew in the oat crops sown round the meteorological ation at the Department of Plant Pathology, Auchincruive.



ext-fig. 1. Relation between the intensity of *Erysiphe graminis* and the number of hours 100 % R.H. per week. Vertical columns represent the number of hours per week with saturated atmosphere (left-hand vertical scale). The full black line shows the development of mildew (right-hand vertical scale).

Measurements of spore germination in atmospheres maintained at andard relative humidities confirm this dependence of the fungus upon tmospheric saturation. Conidia from mildewed oat leaves were shaken on sterile cover-slips, which were stored in sealed glass tubes above solutions ontaining varying concentrations of calcium chloride. A drop of water ontaining conidia was placed on a cover-slip in each series, and this was milarly stored over water to prevent evaporation. The whole series was placed in an incubator at 22° C. Several repetitions were made, and the collected results are given in Table 2. Spores of the fungus *Cladosporium herbarum* are always mixed with those of *Erysiphe graminis* in natural infections, and their differing responses to R.H. are noteworthy.

Table 2. *Germination of* Erysiphe graminis *conidia*

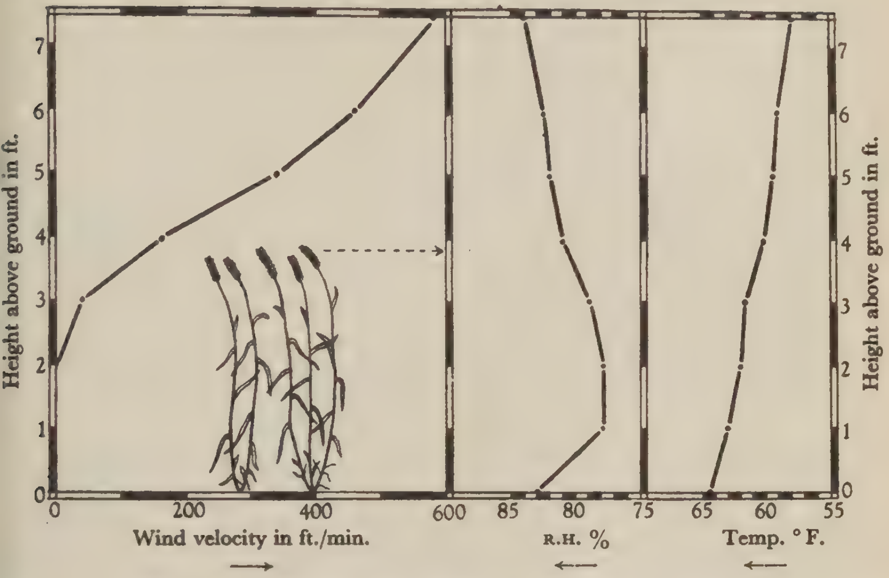
	% R.H.					
Storage conditions	Water	100	90	85	76	66
Maximum percentage germination *Erysiphe graminis*	8	47	1	0	0	0
Germination, *Cladosporium herbarum*	++	++	+	+	+	0
Solution to give storage conditions	Water	Water	15% $CaCl_2$	20% $CaCl_2$	30% $CaCl_2$	40% $CaCl_2$

Spores of the oat strain of *E. graminis* under test germinate much better in air at 100% R.H. (Pl. VA) than in actual water, whilst germination does not take place at relative humidities of 85% or less. Spores of *Cladosporium herbarum* germinate copiously in water and 100% R.H., and grow well at relative humidities down to 76%, but not at 66%. Spores of *Erysiphe graminis* did not grow beyond the stage shown in Pl. VA in these germination tests, but it is noteworthy that *Cladosporium herbarum* grew and produced chains of conidia in water, and over all relative humidities down to 76%.

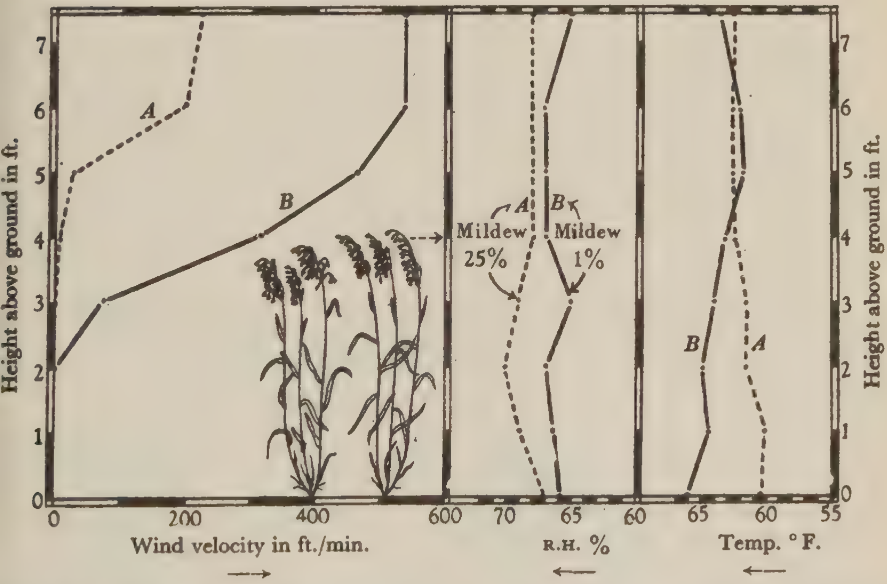
It is reported from Canada (Cherewick, 1944) that conidia of the oat strain of *Erysiphe graminis* will germinate at 100% R.H. and also at 0% R.H. over barium perchlorate. The Scottish strain clearly has not this wide toleration.

E. graminis will attack young oat plants twenty days after germination, if the atmosphere is saturated for sufficient periods. It will also grow upon old leaves, as long as they are green, but ceases to produce conidia when the foliage turns brown. This is apparently not related to the degeneration and loss of the chlorophyll, for no significant germination was obtained when conidia of *E. graminis* were dusted over drops of oat-leaf extract, of green sycamore-leaf extract and anthocyanin-containing red sycamore-leaf extract. Oat-leaf extract, when filtered through filter-paper, gives a cloudy suspension of chlorophyll; sycamore leaves provide a clear, opalescent green or red fluid when similarly prepared. Chlorophyll and anthocyanin therefore appear to be without effect on the germination of *Erysiphe* conidia. It is also interesting that *Podosphaera Oxyacanthae* (DC.) de Bary was found growing on white leaves of hawthorn at Auchincruive in 1945.

Mildew is often found more plentifully towards the base of a cereal crop, than among the upper foliage. This presumably reflects the higher R.H. nearer the ground. It is, indeed, frequently found that humidity is highest near the ground (e.g. in wheat, Text-fig. 2), or in the lower parts of the crop (e.g. oats in Text-fig. 3). These layers presumably reach atmospheric saturation before the air above, and retain it for a longer period. An interesting comparison was possible between two crops of oats grown near one another at Auchincruive in 1946. One crop (*B* in Text-fig. 3) was



ext-fig. 2. Typical meteorological conditions within a wheat crop, 10 Aug. 1945. The wheat had 5 % of mildew in the lowest eighteen inches, and none above; note the highest R.H. near the ground.



ext-fig. 3. Typical meteorological conditions within two neighbouring oat plots at Auchincruive 6 Aug. 1946 (see text).

planted near the top of a low hill, and had an average of 1 % mildew. The other was 300 yards to the south-east, and lay in a slight hollow, to leeward of the hill in relation to the prevailing wind. It experienced much lower wind velocity (*A*, Text-fig. 3), with about 3 % more R.H. than the other crop, and also had 25 % mildew.

It would seem, from general observations, that corn mildew is generally more severe in the west of Scotland than in most parts of England. Heavy attacks occur in all regions, but when I moved from Yorkshire to the west of Scotland I obtained a strong impression that mildew is much more generally severe in the latter region. Data compiled from the Air Ministry's *Monthly Weather Reports* (1938–44) for the various meteorological districts of the British Isles show that the west of Scotland has a large number of rain days per annum, and a slightly higher average R.H. than most regions of England. Differences of average R.H. are not large, but are fairly significant (e.g. $p =$ between 0·05 and 0·02 by *t*-test when comparing the west of Scotland (83 %) with the midlands of England (78 %)). The west of Scotland, moreover, has significantly higher average monthly humidities from May to August, when cereal crops make most growth, than districts in England. Figures of R.H. during the summer months for the west of Scotland are only exceeded by the north of Scotland, where mildew is also severe.

These field and regional studies all tend to confirm the relation between severity of *Erysiphe graminis* and a high R.H. They also point to the need for a more detailed and comparative estimation of disease intensity between regions.

THE EFFECTS OF MAJOR SOIL NUTRIENTS

Most workers report increasing severity of mildew when nitrogenous manures are added to the soil. Field experiments at Auchincruive in 1945 and 1946 suggest that phosphate reduces the severity of mildew and potash increases it. These experiments were carried out in conjunction with Mr John McFarlan of the Department of Agriculture, West of Scotland Agricultural College. The results are shown in Tables 3 and 4.

They show that both nitrogen and potash tend to make mildew more severe, while phosphate reduces its severity. The effect of nitrogen has been confirmed by adding varying amounts of nitrogen to water cultures of oats (Table 5).

Additions of phosphate to land which is already well supplied with phosphate appear to be without effect upon the severity of mildew, when oats are grown (Table 6). It is probably only the correction of actual phosphate deficiency which will reduce the severity of mildew.

Various practical indications suggest that continued applications of artificial manures have an adverse effect upon the severity of mildew. This can be seen by comparing the plot totals for 'no addition' and 'complete manuring' in Tables 3 and 4. The question has also been tested by solution culture, using two methods:

(1) Varying the concentration of solution directly (curve *A* in Text-figs. 4, 5 and 6).

(2) As (1), but the solution renewed every fourteen days (curve *B*).

Table 3. *Soil factors and* Erysiphe graminis, 1945

(Phosphate-deficient soil, Peel Hill, Auchincruive)

Treatment	Mildew, % of leaf area	Plot totals			
o addition	4				
itrogen	19				
hosphate	0·5	Nitrogen	52	No nitrogen	14·5
otash	9	Phosphate	17·5	No phosphate	49
itrogen, phosphate	2	Potash	41	No potash	25·5
hosphate, potash	1	No addition	4	Complete manuring	14
itrogen, potash	17				
itrogen, phosphate, potash	14				

Table 4. *Soil factors and* Erysiphe graminis *on Victory Oats*, 1946

(Soil low in phosphate, Donald's Thorn, Auchincruive)

Treatment	Mildew % of leaf area		Plot totals			
	Block I	Block II				
o addition	22	15				
itrogen	27	30				
hosphate	15	15	Nitrogen	226	No nitrogen	171
otash	42	25	Phosphate	180	No phosphate	222
itrogen, phosphate	40	18	Potash	210	No potash	182
hosphate, potash	20	17	No addition	37	Complete manuring	55
itrogen, potash	33	28				
itrogen, phosphate, potash	28	27				

Manures applied at rates per acre, in 1945 and 1946

itrogen	3 cwt.	Nitro chalk
hosphate	6 cwt.	18% superphosphate
otash	2 cwt.	60% muriate of potash

Table 5. *Nitrogen level and* Erysiphe graminis *on Star Oats*

Culture solution	Mildew % of leaf area average of all leaves
Sachs's + micro-solution: with ½ normal N	7
with normal N	17
with 2 times normal N	25
with 4 times normal N	45

Table 6. *Phosphate level and* Erysiphe graminis *on Star Oats*

	Original cont. of P_2O_5 mg./100 g. of dry soil		Mildew % of leaf area
Soil A	27	No addition	37
		+ Superphosphate	34
Soil B	12	No addition	57
		+ Superphosphate	50
Soil C	0	No addition	5
(phosphate fixing)		+ Superphosphate	0

Each pair of soils was compared separately, and at different times, so that the amounts of ildew recorded are only comparable as between no addition and the application of superhosphate in each case.

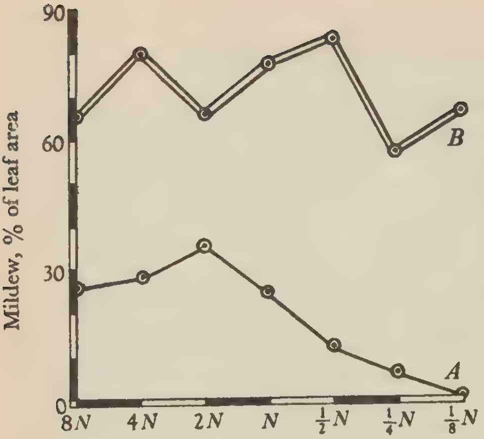


Fig. 4.

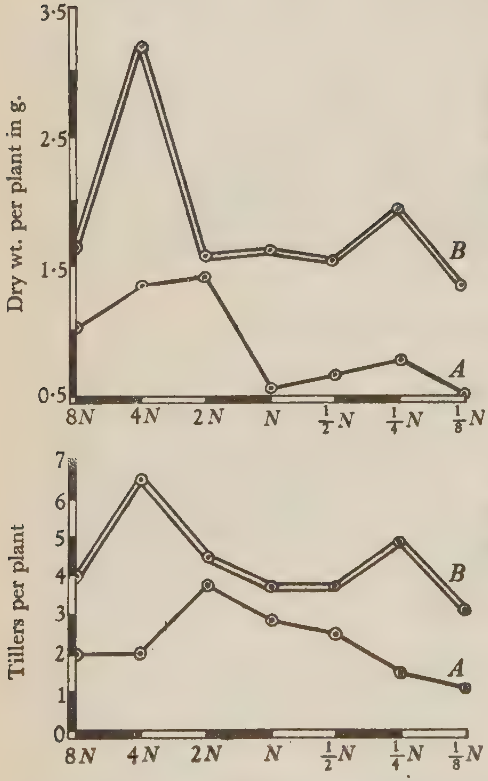


Fig. 5.

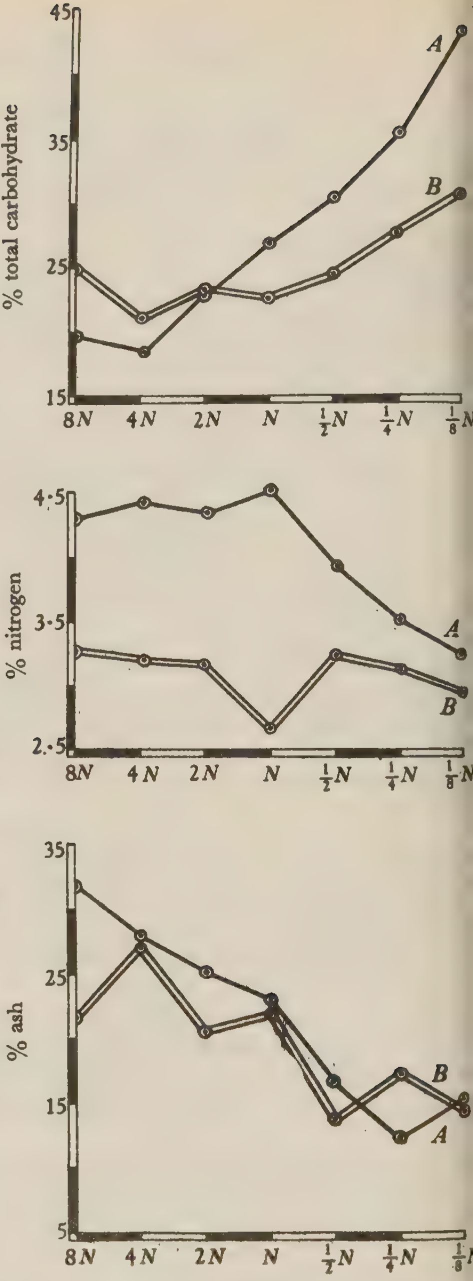


Fig. 6.

Text-fig. 4. Disease incidence of *Erysiphe graminis* at different levels of inorganic nutrition in solution culture. A = original solution retained throughout. B = solution changed every fourteen days. N on the horizontal axis is normal Sachs's solution with micro addition.

Text-fig. 5. Data for dry weight and tillering of Star oats grown at different levels of inorganic nutrition in solution culture. A = original solution retained throughout. B = solution changed every fourteen days. N on the horizontal axis is normal Sachs's solution with micro addition.

Text-fig. 6. Data for total carbohydrate, nitrogen and ash at different levels of inorganic nutrition in solution culture. A = original solution retained throughout. B = solution changed every fourteen days. N on the horizontal axis is normal Sachs's solution with micro addition. Percentages of nutrients are expressed on a dry-weight basis.

Sachs's solution with additions of micro-elements was made up in seven oncentrations, from one-eighth normal to eight times normal. Five culture ars were set up for each strength. Results of dry weight and of tillering re given in Text-fig. 5 and of the incidence of mildew in Text-fig. 4. They how that changing the solution every fourteen days (*B*) resulted in inreased dry weight and increased tillering, as compared with the retention f the same solution throughout (*A*). Mildew was much more severe in eries *B* (Text-fig. 4), where the solution was changed every fourteen days. The incidence of mildew also increased with increasing concentration in eries *A* (Text-fig. 4) up to twice the normal concentration. This provides ontributory evidence that raising the level of inorganic manuring brings ncreased incidence of mildew.

THE EFFECT OF COMPOSITION OF THE HOST PLANT

Differences between the amounts of mildew with varying treatment in the ast experiment seemed to provide the possibility of a good test as to whether the amounts of carbohydrate, Kjeldahl nitrogen and ash present n the host had any effect upon the severity of mildew.

All the five plants of each treatment were accordingly dried at 98° C. and were subsequently analysed for total carbohydrate by the Willaman and Davidson (1924) method, for nitrogen by Kjeldahl technique, and for ash by incinerating in a muffle furnace. The results are portrayed in Text-fig. 6, which shows that no relation appears to exist between the amounts of carbohydrate and ash and the level of disease (compare with Text-fig. 4). Total nitrogen is low in series *B*, which has severe mildew, and s higher in series *A*, with much less disease. This is rather surprising, in view of the increase in severity of *Erysiphe* with unbalanced nitrogenous manuring. It suggests that the increase of disease by nitrogenous manuring s brought about rather by an indirect effect of the nitrogen upon the anatomy of the plant, than by its amount within the host.

Another method of testing consists in growing plants under varying lengths of day. It was noticed that spring-sown wheat being grown under eight-hour day conditions developed mildew, whereas the same crop under normal illumination did not do so, nor did autumn-sown wheat under any condition of illumination in 1945. This material was also examined for total carbohydrate, nitrogen and ash, with results set forth in Table 7. The strain of mildew was not that used in the foregoing experiments, but the results are nevertheless useful. They show very little difference in carbohydrate content between the four treatments, but there is more ash and also more nitrogen in the plants which developed mildew. The association of higher nitrogen with severe disease is in contrast with the results of the last experiment, and it was thought that the higher nitrogen might reflect the delay in ripening of the spring-sown, short-day plants. That phenology is not the whole explanation of attack by mildew appeared in 1946, when the disease again developed on short-day oats and wheat, but not so severely on the plants grown with normal length of day, except that infection of the wheat became more general in August. This appearance of the disease occurred in the early spring, when the autumn-sown wheat had leaves only a few inches long. Mildew is also recorded on wheat in June and on wheat and oats in August. The oats had green leaves in both short-day and long-day settings in August, so there is here no question of ripeness preventing attack by mildew. The collective results shown by Text-figs. 4 and 6 and Tables 7 and 8 do not furnish any correlation between the severity of attack by *Erysiphe graminis* and the major constituents of the host.

Table 7

			Total %					
		Date of flg.	Height ft. in.	Total D.W. grams	% mildew	% total carbo-hydrate in leaves	% ash	% N
LD AS	Sq. Master	21 June	3 2	13·64	0	31·43	6·25	0·78
	White Victor	22 June	3 3	10·55	0	31·45	7·70	0·72
	Als	24 June	3 1	12·98	0	35·78	3·43	0·54
LD SS	Sq. Master	29 June	2 10½	13·60	0	32·91	6·09	0·77
	White Victor	30 June	2 9	9·53	0	41·46	6·52	0·59
	Als	4 July	2 8	12·00	0	44·50	8·40	0·59
SD AS	Sq. Master	18 July	2 3	9·26	0	31·45	3·79	1·01
	White Victor	20 July	2 3	6·21	0	39·03	9·40	1·19
	Als	20 July	1 11	7·16	0	33·03	8·21	1·15
SD SS	Sq. Master	*	10	2·80	5	38·59	11·39	1·28
	White Victor	*	11	2·71	16	41·43	9·77	1·47
	Als	*	7	6·43	2	32·17	10·37	1·19

Samples for analysis were taken 20 August 1945
* Did not flower

Table 8

					% mildew					
		Date of flg	Height ft. in.	% solids	10/3	11/6	5/8	% total carbo-hydrate	% ash	% N
Star oats	LD SS	16 July	2 3	23·5	—	0	0·5	41·6	10·0	2·48
	SD SS	—	11	21·7	—	0	7	42·0	8·2	3·84
Atle wheat	LD AS	24 June	2 0	27·1	0	0	5	39·6	7·9	1·93
	SD AS	—	1 6	24·9	10	5	5	42·0	4·6	2·17
White Victor	LD AS	24 June	2 0	29·1	0	1	40	38·9	7·9	2·25
wheat	LD SS	15 Aug.	1 10	22·3	—	0	1·5	40·9	7·6	2·00
—	SD AS	—	1 9	25·3	15	22	28	36·2	11·9	2·54
—	SD SS	—	6	19·5	—	0	30	28·6	12·0	3·65

Samples for analysis were taken 5 August 1946

LD Long day (normal variation in length of day)
SD Short day (eight hours exposure to light)
AS Autumn sown (Table 7) 23 Sept. 1944, (Table 8) 14 Nov. 1945
SS Spring sown (Table 7) 28 Feb. 1945, (Table 8) 12 Apr. 1946
— Not sown on 10 March 1946

It is also interesting that mildew on oak, wild cherry and rose does not appear to be correlated with the amounts of total carbohydrate, or with the content of reducing sugars (Table 9). These results are from material taken for analysis on 11 August 1936.

Table 9

	Solids % of fresh wt.	Total carbohydrate % of dry wt.	Reducing sugars % of dry wt.
A. *Microsphaera Alni* on oak (*Quercus robur*), Dean Nick, Huddersfield			
Healthy young leaves	29·25	38·93	33·07
Mildewed young leaves	34·57	14·71	13·65
Healthy old leaves	41·70	13·45	5·27
B. *Podosphaera Oxyacanthae* on wild cherry (*Prunus padus*), Dean Nick, Huddersfield			
Healthy young leaves	28·74	19·85	17·54
Mildewed young leaves	28·29	19·52	19·52
Healthy old leaves	32·28	10·00	10·00
C. *Sphaerotheca pannosa* on cultivated rose, Huddersfield			
Healthy young red leaves	24·5	34·21	25·26
Healthy young green leaves	36·4	25·78	24·37
Green-red leaves with mildew	30·0	26·62	24·93
Green-red leaves; mildew removed	28·7	20·12	20·12
Healthy old leaves, 1	37·9	16·88	16·88
Healthy old leaves, 2	26·5	30·95	29·29

SUMMARY

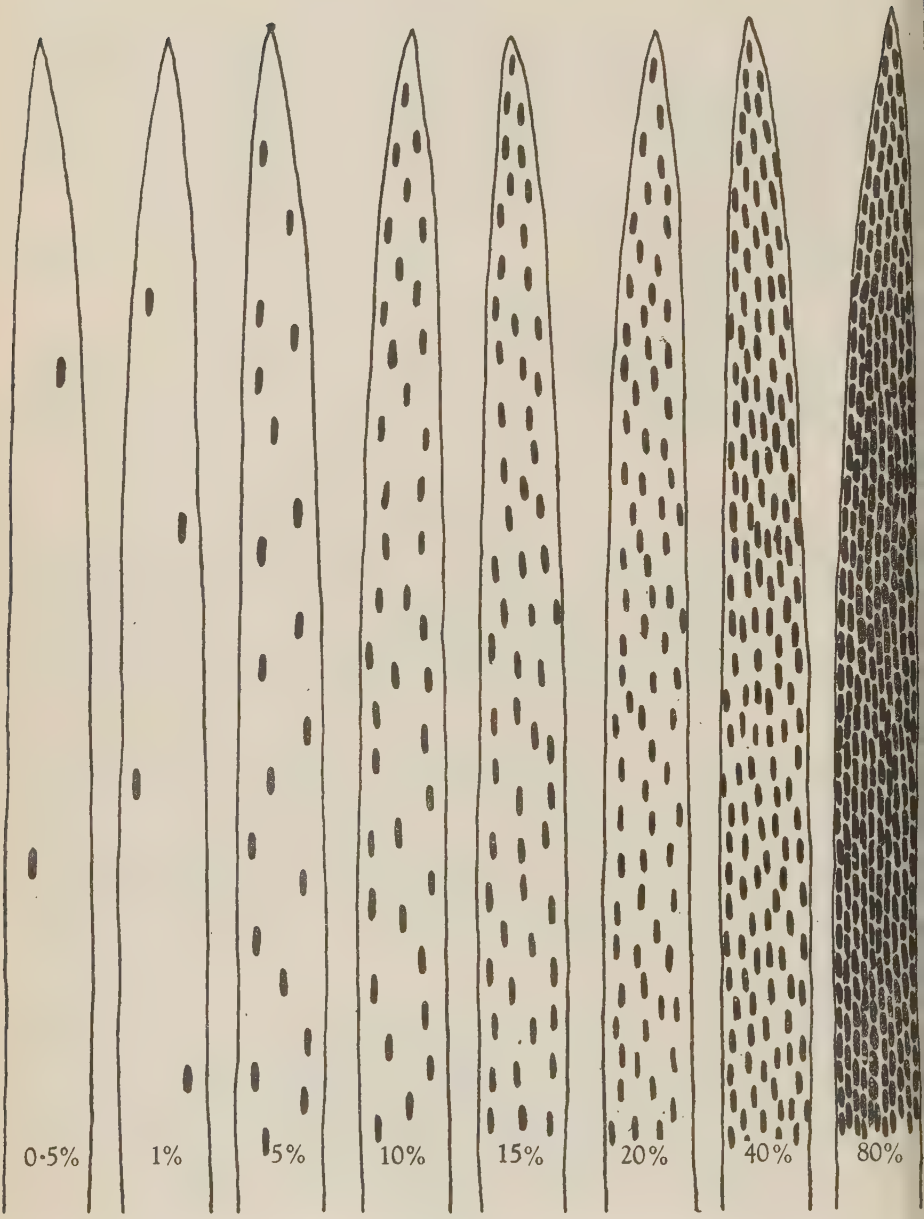
. strain of *Erysiphe graminis* which attacks oats in the west of Scotland has een mainly used in the present investigation. Conidia germinate much etter in a saturated atmosphere than they do in water. Germination is ow at 90 % R.H. and apparently does not take place at lower values of R.H. pores of *Cladosporium herbarum* which accompany those of *Erysiphe graminis* ill grow and fruit in water and at humidities down to 76 %, but not ower.

Climatic correlations suggest that the severity of the disease in the field is elated to the degree of saturation of the atmosphere. Regional and field udies support this view. Severity of the disease appears to owe much iore to the inorganic nutrition than to the gross composition of the host. ractical control of the disease in the west of Scotland lies in the avoidance f excess nitrogen and potassium in the soil, the correction of any deficiency f phosphate, and the avoidance of heavy and frequent application of rtificial manures.

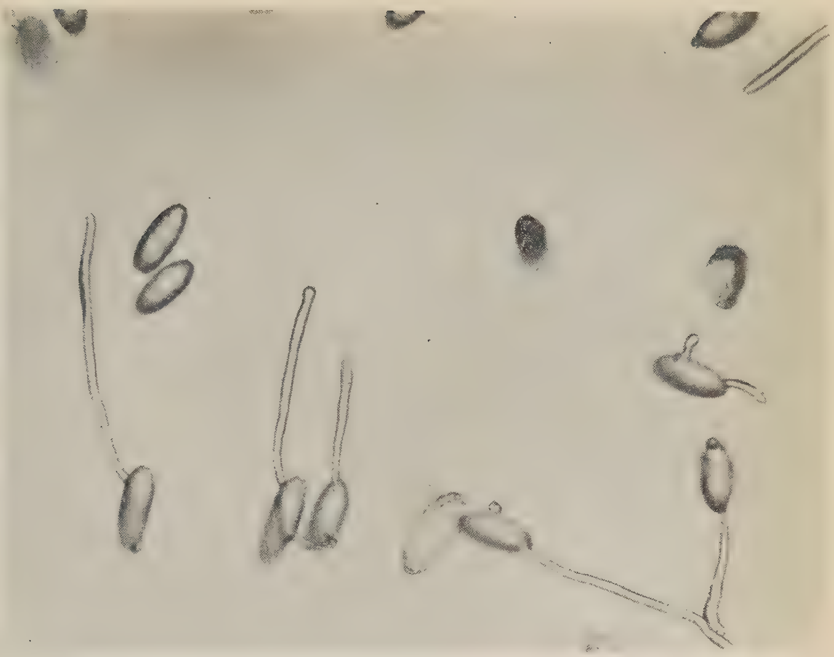
The writer wishes to express his grateful thanks to members of his staff t the Department of Plant Pathology, Auchincruive, for their general ssistance, and to Mr H. F. Dovaston for the photomicrographs of Plate IV. Ir John McFarlan has maintained valued collaboration with the field xperiments, and the determinations of Table 9 were made in 1937 with ie help of Mr A. Broadbent of Huddersfield.

APPENDIX

ı this paper the severity of mildew has been expressed as a percentage of ie total leaf area of the host which was covered by the fungus. This has een accomplished conveniently by comparing all leaves of the plant with tandard area' diagrams (Text-fig. 7). An average of all these readings gave



Text-fig. 7. The 'standard area' diagrams used for the determination of percentage leaf area affected by *Erysiphe graminis*.



A

B

e disease measurement for the plant as a whole. In field measurements it as found adequate to assess the disease intensity at the base, middle and p of the crop, the average of all three being the reading for the sampling ation. Four stations were estimated on plots $\frac{1}{40}$ acre in area, nine in acre plots, whilst twenty sampling stations were measured in each field, hen it was necessary to make more widespread estimations.

The 'standard area' diagrams were made by spacing ovoid dots upon leaf-shaped area, the dots having standard percentages of the leaf area. hey thus provide a standard of comparison for different persons, for fferent regions and for different times. It does not seem to matter very uch that the standard areas can never equal those of any leaf in actual ape and distribution; the human eye detects fairly readily the similarity distribution frequency and pattern.

REFERENCES

HEREWICK, W. J. (1944). Studies on the biology of *Erysiphe graminis* DC. *Canad. J. Res.* XXII, 52–86.

ILLAMAN, J. J. & DAVIDSON, F. R. (1924). Some modifications of the picric acid method for sugars. *J. agric. Res.* XXVIII, 474–88.

EXPLANATION OF PLATE V

(above). Germination of conidia of *Erysiphe graminis* in air at 100% R.H. B (below). Conidia in air at 90% R.H. Only one spore has germinated, and many spores are shrivelled.

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SPONTANEOUS VARIATION IN *PENICILLIUM NOTATUM* STRAIN N.R.R.L. 1249 B21

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(With Plates VI and VII and 1 Text-figure)

Since certain strains of *Penicillium notatum* had been reported to be very variable and much variation had been observed in the cultures used for the commercial production of penicillin, it was decided to investigate this phenomenon. The strain examined most thoroughly was N.R.R.L. 1249 B21, which was distributed by the Northern Regional Research Laboratories, Peoria, Illinois, and has been widely used for commercial production by the surface culture method. It was derived by successive selections from the original Fleming strain. Raper and Alexander (1945) have described this strain and given an account of its history. They reported the occurrence of paler, less highly sporulating variants and of darker, more heavily sporulating variants. The present investigations were begun before the publication of Raper and Alexander's paper, and where the observations coincide the results obtained are in agreement with those of Raper and Alexander. Variants showing differences in sporulating capacity have been obtained by single conidial platings and by streaking from mycelial patches on old cultures. The poorer sporulators were found to be the higher penicillin yielders up to a certain limit. Very poor sporulators, however, seem to be very poor yielders of penicillin as reported by Raper and Alexander (1945).

It is the purpose of this paper to deal in greater detail with the occurrence of variations affecting sporing capacity, with a view to elucidating the cause or causes of the variation.

METHODS

Streaking from old cultures

Variants were obtained from patches of mycelial growth overgrowing the normal conidial type on old cultures. By streaking and finally inoculating on to plates of plain agar it was usually possible to obtain single hyphal isolations of these types. These gave apparently pure cultures of mycelial habit. Cultures obtained in this way I have called 'mycelial types'. Similar 'mycelial types' were also obtained by conidial platings of old cultures.

Single conidial isolations

Variants were also obtained by plating out suitable dilutions of conidial suspensions taken from cultures of various ages. The conidial suspensions were made in physiological saline and detergent (0·1 % calsolene oil). The suspensions were well shaken to break up chains of conidia and filtered through absorbent cotton under sterile conditions. This removes pieces of mycelium and clumps of conidia. The density of a conidial suspension was ound by making haemocytometer counts and from this suitable dilutions ere estimated and plated. The haemocytometer observations also per- itted a check on the presence or not of clumps of conidia. If such were bserved the suspension was shaken and re-filtered. This was very seldom ecessary, the conidia being usually well separated. A small amount 0·2 c.c.) of the required spore dilution was spread over the surface of an gar plate with a spreader made of bent glass tubing. It was preferred to pread the spores over the surface of the agar rather than to mix them in ooled melted agar and then pour them, since the former method gave more regular germination of spores which facilitated a comparison of the ngle spore colonies.

With some exceptions the medium used was 3 % malt extract with 2 % gar. On this medium the types of variants studied were clearly differ- ntiated from each other and the parental type.

PRESENT OBSERVATIONS

n ampoule of *Penicillium notatum* N.R.R.L. 1249 B21 (circulated by the orthern Regional Research Laboratories, Peoria, Illinois), was opened nd subcultured. The subculture was transferred to plain agar and single yphal isolations made. These proved to be of two types, indicated here as 1 and T2. Type 1 (probably the parental 1249 B21 type) was paler and ss heavily sporulating than T2. It is not known whether the colour ifference between the two types is entirely due to difference in conidia roduction or not. T2 also differed from T1 in that it produced less ellowing of the medium.

On malt agar T1 gave a smooth velvety growth, pea green (Pl. 47, idgway colour chart) on the eighth day. T2 gave a similar type of growth, ge green (Pl. 47) on the eighth day. On the modified Czapek agar used by aper and Alexander, when three colonies were inoculated on one plate, e edge of the plate was reached by the tenth day. T2 showed a rather at velvety growth with a few shallow radial furrows. T1 was similar to T2 ith slightly more pronounced furrows. The colours on the tenth day ccording to the Ridgway colour chart were artemisia green (Pl. 47) at e outside, shading to Hathi grey (Pl. 52) in the centre for T1, and temisia to celandine green at the edge, shading to dark olive grey in the ntre for T2. In both cases the colony reverse was yellow.

Colonies of each type were more uniform and more distinct from the her type on malt than on Czapek agar.

At first it was not known whether T1 or T2 was the standard 1249 B21 pe. However, according to the description given by Raper and Alexander, 1 would appear to correspond to the original type, T2 probably being variant derived from it.

Conidial platings were made from the original single hyphal isolates, and ngle conidium cultures were obtained by transferring a piece of agar con- ining a single germinated conidium on to an agar slant under the dissecting icroscope. Spores from the single spore cultures were in time plated out d in this way data were obtained concerning the number of variant lonies given by single hyphal and single conidium cultures.

Table 1. *Production of pale and dark variants by H.T. (single hyphal) and S.S. (single conidium) cultures of T 1 type*

	Total colonies	Dark	% dark	Pale	% pale	Other types	% other types	Total variants	% variants
*H.T.: 11 days	179	1	0·6	3	1·7	—	—	4	2·2
23 ,,	775	10	1·3	6	0·8	5	0·6	21	2·7
28 ,,	243	5	2·1	1	0·4	—	—	6	2·5
42 ,,	134	2	1·5	—	—	—	—	2	1·5
10 ,,	621	4	0·6	17	2·7	—	—	21	3·4
10 ,,	534	4	0·7	5	0·9	—	—	9	1·7
18 ,,	510	2	0·4	13	2·5	3	0·6	18	3·5
Total	2996	28	0·9	45	1·5	8	0·3	81	2·7
*S.S.: 13 days	401	7	1·7	11	2·7	—	—	18	4·5
4 ,,	696	—	—	5	0·7	—	—	5	0·7
21 ,,	253	5	2·0	—	—	—	—	5	2·0
10 ,,	1117	4	0·4	18	1·6	—	—	22	2·0
13 ,,	1092	7	0·6	21	1·9	—	—	28	2·6
Total	3559	23	0·6	55	1·5	—	—	78	2·1
Total H.T. and S.S. cultures	6555	51	0·8	100	1·5	8	0·1	159	2·4

Table 2. *Production of pale and dark variants by H.T. (single hyphal) and S.S. (single conidium) cultures of T 2 type*

	Total colonies	Dark	% dark	Pale	% pale	Other types	% other types	Total variants	% variants
*H.T.: 10 days	324	1	0·3	—	—	—	—	1	0·3
10 ,,	2268	—	—	—	—	—	—	—	—
10 ,,	147	6	4·1	—	—	—	—	6	4·1
* 11 ,,	529	2	0·4	—	—	—	—	2	0·4
18 ,,	626	—	—	—	—	—	—	—	—
28 ,,	626	—	—	4	0·6	—	—	4	0·6
* 11 ,,	560	—	—	4	0·7	—	—	4	0·7
42 ,,	239	—	—	—	—	1	0·4	1	0·4
* 42 ,,	199	2	1·0	1	0·5	—	—	3	1·5
Total	5518	11	0·2	9	0·2	1	—	21	0·4
S.S.: 4 days	493	—	—	—	—	—	—	—	—
10 ,,	2154	—	—	—	—	—	—	—	—
13 ,,	782	—	—	22	2·8	—	—	22	2·8
* 13 ,,	468	6	1·3	1	0·2	—	—	7	1·5
21 ,,	361	3	0·8	1	0·3	—	—	4	1·1
* 29 ,,	648	4	0·6	5	0·8	—	—	9	1·4
* 29 ,,	199	—	—	—	—	—	—	—	—
Total	5105	13	0·3	29	0·6	—	—	42	0·8
Total H.T. and S.S. cultures	10,623	24	0·2	38	0·4	—	—	63	0·6

* Obtained from cultures used in production at the I.C.I. plant in Trafford Park.

The results obtained from cultures of T 1 type are shown in Table 1 and those from darker types in Table 2. These tables include a number of dark and light selections obtained from cultures used in production at the I.C.I. plant in Trafford Park. The cultures within one group were not identical in appearance and it is possible that they differed by certain genetic modifying factors. Table 1 shows the results obtained by plating seven single hyphal and five single conidium cultures of Type 1. There were no statistically significant differences between platings from the single hyphal and single conidium cultures. There were 0·8 % of darker variants, 1·5 % of paler variants and 0·1 % of other variant types, 2·4 % in all.

The darker types (Table 2) gave only 0·6 % of variants, 0·2 % of darker variants and 0·4 % of paler variants. The cultures of T 1 gave approximately four times as many variants as those to T 2. This spore plating method for detecting variants might be expected to favour the higher sporulating types but since this would tend to increase the number of darker variants and decrease the number of paler variants recorded in both T 1 and T 2, it is possible that the darker types are actually more stable than the paler ones. It is also possible that the cultures included in Table 2 are not homogeneous with respect to rate of variation, some being more stable than others. Very stable strains have been obtained from T 1. On one occasion a very dark heavily sporulating type giving excessive yellowing of the medium was obtained from a culture of T 1. This proved to be very stable and paler variants derived from it were also relatively stable.

The preliminary investigations showed a remarkable degree of uniformity both in the proportions of mutants and in their appearance. It seemed possible therefore that the greater part of the variability observed in this strain might be due to the constant occurrence of a particular type of change rather than to random variations of different origin. In order to test this hypothesis T 1 was studied more intensively.

Analysis of Type 1 *variants*

From a plating of T 1, a standard T 1, a pale variant T 1 *a*, and a dark variant colony T 1 *b*, were streaked and finally single hyphal isolations were made. Conidia from these isolates were plated and single conidium isolations were made. It was found that T 1 *a*, gave a still paler variant type, T 1 a_2 and also variants resembling T 1 and T 1 *b*. This seems to indicate that changes in sporulating capacity occur in steps since T 1 *a* gives a still paler variant T 1 a_2 and that these changes are reversible since T 1 *a* gives a type closely resembling T 1. The results from seven single conidium cultures of T 1 are given in Table 3, and from eleven single spore cultures of T 1 *a*, in Table 4. It will be noticed that every culture gave colonies of a paler type, T 1 *a* and that all except two gave a darker type, T 1 *b*. The percentages of these variants were remarkably constant from one culture to another, with the exception of S.S. 3 which was recorded as having a visible pale sector and which gave a higher percentage of pale variants than its sister cultures. The average percentage of T 1 *b* variants was 0·4 and of T 1 *a*, excluding the culture with a visible pale sector, 2·2. The percentage of variants of all types was 2·7, excluding S.S. 3. Single conidium cultures of the pale type T 1 *a* gave 0·9 % of still paler variants, T 1 a_2, 1·7 % of variants resembling T 1 and 0·3 % of variants of T 1 *b* type, as shown in Table 4. Again the percentages of variants of the different types were very constant among the offspring of the sister cultures. Only in one culture, which had been recorded as having a visible green sector, was there any striking deviation from the average percentages. The average percentage of total variants, excluding the culture with a sector, was 3·0, not significantly different from the 2·7 observed in the case of T 1. Only four cultures of

Table 3. *Production of dark (T 1 b) and pale (T 1 a) variants by sister single conidium cultures of T 1 type*

Culture	Total colonies	T 1 *b* variants	% T 1 *b*	T 1 *a*	% T 1 *a*	Others	%	Total variants	% variants
S.S.: (1) 12 days	967	—	—	13	1·3	—	—	13	1·3
(2) ,, ,,	553	1	0·2	12	2·2	1	0·1	14	2·5
* (3) ,, ,,	1100	4	0·4	97	8·8	—	—	101	9·2
(4) ,, ,,	1064	4	0·4	26	2·4	1	0·1	31	2·9
(5) ,, ,,	353	—	—	4	1·1	1	0·3	5	1·4
(6) 19 ,,	486	9	1·9	11	2·3	—	—	20	4·1
(7) ,, ,,	504	2	0·4	26	5·1	—	—	28	5·6
(8) ,, ,,	562	4	0·7	6	1·6	—	—	10	1·8
Total	5589	24	0·4	195	3·5	3	0·1	222	4·0
Total excluding S.S. (3)	4489	20	0·4	98	2·2	3	0·1	121	2·7

* Recorded as having a visible pale sector.

Table 4. *Production of dark (T 1 and T 1 b) and pale (T 1 a_2) variants by sister single spore cultures of T 1 a type*

	Total colonies	T 1 variants	% T 1	T 1 *b* variants	% T 1 *b*	T 1 a_2 variants	% T 1 a_2	Total variants	% total
S.S.: (1) 12 days	485	3	0·6	1	0·2	8	1·7	12	2·5
(2) ,, ,,	355	5	1·4	—	—	3	0·9	8	2·3
(3) ,, ,,	1049	12	1·1	2	0·2	6	0·6	20	1·9
(4) ,, ,,	833	11	1·3	2	0·2	9	1·1	22	2·6
(5) ,, ,,	1024	10	0·9	1	0·1	13	1·3	24	2·3
(6) 19 ,,	330	3	0·9	1	0·3	8	2·4	12	3·6
(7) ,, ,,	474	11	2·3	4	0·8	1	0·4	16	3·4
(8) ,, ,,	970	18	1·9	2	0·2	5	0·5	25	2·6
* (9) ,, ,,	825	159	19·3	3	0·4	5	0·6	167	20·2
(10) ,, ,,	911	37	4·1	8	0·8	6	0·7	51	5·6
(11) ,, ,,	429	8	1·9	1	0·2	6	1·4	15	3·5
Total	7685	277	3·6	25	0·3	70	0·9	372	4·8
Excluding S.S. (9)	6860	118	1·7	22	0·3	65	0·9	205	3·0

* Recorded as having a visible dark sector.

Table 5. *Production of darker (T 1 b_2) variants by cultures of T 1 b type*

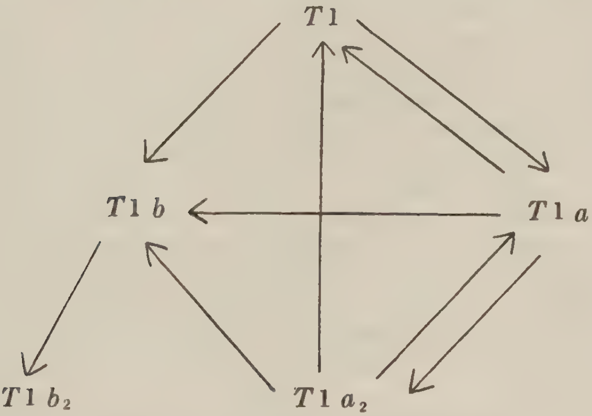
Culture	Total colonies	T 1 b_2 variants	% T 1 *b* variants
H.T. (42 days)	1029	—	—
S.S. (1) (14 days)	691	1	0·1
S.S. (2) (14 days)	981	28	2·9
S.S. (3) (14 days)	909	—	—
Total	3610	29	0·8

Table 6. *Production of darker (T 1 a, T 1 and T 1 b) variants by cultures of T 1 a_2 type*

Culture	Total colonies	T 1 *a* variants	% T 1 *a*	T 1 variants	% T 1	T 1 *b* variants	% T 1 *b*	Total variants	% variants
S.S. (1) 40 days	51	3	5·9	3	5·9	2	3·9	8	15·7
S.S. (2) 11 days	229	32	14·0	6	2·6	7	3·1	45	19·7
Total	280	35	12·5	9	3·2	9	3·2	53	18·9

T 1 *b* were tested (Table 5). Two of them gave no variants and the other two gave variants of a still darker type, T 1 b_2. The average percentage of these mutants was 0·8 % but all except one came from one culture so this ercentage may be higher than that which would be obtained if more ultures were tested. The darker variant T 1 b_2 has not been further in-estigated.

Two cultures of type T 1 a_2 have been plated and each gave variant olonies of T 1 *a*, T 1 and T 1 *b* types. T 1 a_2 appears to be the most unstable pe so far encountered since it gave an average of 18·9 % of variant olonies. However, since T 1 a_2 produces very few conidia, variants with reater sporulating capacity would be expected to produce more conidia in roportion to the amount of mycelium present. Hence the conidial lating method of testing variation might be expected to exaggerate the ctual variation. However, sectoring was more frequent for T 1 a_2 than for e other types, therefore it is probably actually less stable than the other embers of the series, although probably not to the extent indicated by the ercentage of variant colonies obtained.



Text-fig. 1. Variation of T 1 and derivatives.

The production of variants by the different types is illustrated in ext-fig. 1.

In discussing the tables, it has been assumed that the variants occurring different cultures were similar variants.

An attempt was made to see how far these variants of similar appearance, ccurring regularly in different single spore cultures were identical. treaks were made from variant colonies. Conidial suspensions were made om these streaks and inoculations were made at three places on an agar late as follows, using the paper disc method of inoculation devised by r P. H. Gregory. Two small sterile filter paper discs were immersed in the ore suspension and placed side by side on the agar plate. Two discs from suspension made from a similar type of different origin were placed at second position and one disc from each suspension was placed at the third sition. Variants of T 1 *b* type arising from four different T 1 *a* type ltures were tested against a variant of T 1 *b* type arising directly from T 1. some cases two different colonies from the same type were tested. In addition, two T 1 *b* cultures arising in two different single conidium cultures of T 1 type were tested against the control.

In all these tests the three colonies on one plate were identical in appearance (see Pl. VI, fig. 3).

Three variants of T 1 type from three different single spore cultures of T 1 *a* type were similarly tested and gave identical colonies on each plate. See (Pl. VI, fig. 4).

Variants of T 1 *a* type obtained from four different single conidium cultures of T 1 type were combined with a T 1 *a* type from a fifth single conidium culture. Mass inoculations were used instead of spore suspensions because of the reduced sporulation in the type. The five cultures tested appeared identical (see Pl. VI, fig. 5).

Variants of T 1 a_2 from four different single conidium cultures of T 1 a_1 type tested in the same way appeared to be identical (Pl. VI, fig. 6).

This method, of inoculating cultures of similar appearance side by side, was used, as being the most stringent test of identity available, in the absence of sexual reproduction. Besides affording the possibility of comparing the cultures, in conditions as nearly as possible identical, it also permits of heterokaryon formation at the line of junction of the two types. The use of heterokaryons is at present the only possible test for allelism in Fungi imperfecti. When two different mutants form a heterokaryon it often happens that the mutation in each locus is recessive to its allele on the other nucleus, so that the heterokaryon is different in appearance from its two components. If, for example, two T 1 a_2 types of different origin inoculated side by side gave a green streak at their line of junction it could be deduced that the two types, while phenotypically similar, were genotypically different. However, the absence of such an effect does not prove that they are identical although it is in accordance with such a view.

Description of T 1 variants

Macroscopic. A description is given of the variants on malt agar which was used as the principal medium and also on the modification Czapek agar used by Raper and Alexander (1945). The various types in the T 1 series are most clearly differentiated on malt agar on which they give a smooth velvety growth. T 1 and T 1 *b* show a completely flat growth but T 1 *a* shows very shallow radial furrowing, and T 1 a_2 a more distinct radial furrowing.

The colours of the types according to Ridgeway's colour chart are as follows:

Type	8 days on malt	11 days on malt	10 days on Czapek-Dox
T 1 *b*	Sage green 29'''', Pl. 47	Pea green 29'''', Pl. 47, shading to celandine green 33''''*b*, Pl. 47	Celandine 33'''' through artemisia 33'''', Pl. 47, to deep olive 23''''' or mouse grey 15''''', Pl. 51
T 1	Pea green 29'''', Pl. 47	Court grey 29'''', Pl. 47	Artemisia 33'''', Pl. 47, at edge shading to Hathi grey 35''''' or storm grey 35''''', Pl. 52, in centre
T 1 *a*	Greenish glaucous 33''', Pl. 41	Greenish glaucous 33''' to yellowish glaucous 25''', Pl. 41	
T 1 a_2	Sea-foam yellow 25'', Pl. 31	Sea-foam yellow 25'', Pl. 31	

It will be noticed that colour differences can be observed among the ifferent types on malt agar. On Czapek agar, however, the colours are he same for T 1, T 1 *a*, T 1 a_2, but the central paler regions differ in size, eing largest in T 1 a_2 and smallest in T 1. The differences in macroscopic ppearance seem to be due to the fact that T 1 *b* → T 1 → T 1 *a* → T 1 a_2 form series of types tending to spore later and less freely.

Microscopic. Preparations were made by fixing portions of mycelium in ormol-alcohol for a few minutes, staining in chlorazol black E (saturated olution in 70 % alcohol), rinsing in 70 % and mounting in gum chloral. hlorazol black was used as a fungal stain by Armitage (1944). The use of um chloral as a mountant was suggested by Dr P. H. Gregory. It has an dvantage over balsam and gum damar for this material in that dehydration and clearing in xylol is eliminated. Hence a greater number of enicilli are left, comparatively undisturbed. It is not known how long uch preparations will last but they have been kept for six weeks without ppreciable fading. The material was taken from nine-day cultures except n the case of T 1 a_2. (In this type no penicilli could be found in nine-day ultures so mounts were made from 26-day old cultures.)

T 1. Raper and Alexander (1945) have given a detailed description of he conidial apparatus of 1249 B21 and it is therefore unnecessary to epeat this here. So far as I can determine, T 1 corresponds to 1249 B21 nd the description given for this strain holds for T 1. Metulae vary from ne to four in number, sterigmata from one to five with three as the most requent number and a certain number of irregular penicilli are present Pl. X, fig. 8).

T 1 b (Pl. VII, figs. 7, 11) forms more conidia than T 1 and this is correlated with differences in the number of sterigmata and in the number of conidia o be found on one sterigma. Thus the most frequent arrangement of metulae was in groups of four as compared with three in T 1. Some terigmata were observed with chains of eight conidia attached whereas the greatest number of conidia attached to one sterigma in T 1 was six. It is ealized that the actual number of conidia observed in the chains is likely o be affected by the mounting process but it is felt that the observations give a fair representation of the relative lengths of chains in the different ypes.

T 1 a_1. In T 1 a_1 (Pl. VII, fig. 9) the greatest number of sterigmata observed had only one conidium and the longest chains recorded consisted of only four conidia. Most frequently only one sterigma was present, but perfectly regular penicilli with groups of four sterigmata were observed. However, irregular penicilli were much more frequent in this type than in the two preceding types; T 1 *b* had 1/30 irregular penicilli, T 1, 5/42 and T 1 a_1 14/36.

The irregularities consisted chiefly in the formation of secondary metulae and in a spreading in the position of the sterigmata. The lower-sporulating types tend to have much less compact penicilli than the normal and the various abnormalities seem to be related to this tendency.

T 1 a_2 (Pl. VII, figs. 10, 12) shows a still further reduction in sporulation capacity than T 1 *a*. More penicilli consisting of only one or two sterigmata were present than in T 1 *a*. A characteristic of this variant is that the sterigmata are often flattened. The variation in spore size is much greater than in the other types.

Mycelial types. As previously described, patches of mycelial growth frequently occur on old cultures of *P. notatum*. Such patches have been observed on cultures started from a single conidium and therefore originating from one nucleus. A number of *mycelial* types have been obtained from single conidium cultures by streaking and finally making hyphal tip inoculations. Several such cultures have been isolated from single spore cultures of similar type. These cultures differ from each other in general appearance, in the amount of pigment produced and in colony shape but they are all alike in that they rarely revert to the conidial form. The change from the conidial to the mycelial habit appears to be essentially irreversible. Most mycelial types produce some conidia and it has twice been possible to observe that these conidia are formed on greatly reduced, almost abortive penicilli. However, when these conidia are plated out they produce colonies of mycelial type only. In other characteristics, such as colony shape, the mycelial types are subject to variation. All the mycelial types tested by the surface culture method have been found to be poor yielders of penicillin: this is in accordance with the results of Raper and Alexander. They did not distinguish between mycelial types which could not revert to conidial types and very poor sporulating types which could throw higher sporulating types but it is probable that their 'g' and 'M' types are mycelial types in the sense of this paper. Mycelial types have been obtained from both T 1 and T 2 strains. It is probable that mycelial patches may differ in frequency and extent in different strains but few quantitative data have been obtained on this point.

Mycelial types can also be obtained by single conidium isolations from old cultures. One particular mycelial type with a spiky transparent type of growth has been obtained in this way from four different single spore isolations of T 1 and from one of T 2. One mycelial type, obtained on an old T 1 culture, after continuing unchanged through a number of mass transfers eventually gave a sector of this peculiar transparent type.

DISCUSSION

The evidence presented indicates that T 1 and its derivatives are subject to frequent variation of a particular type. The variation is progressive since T 1 *a* gives a paler type T 1 a_2, and T 1 *b* gives a darker type; and it is reversible to a certain extent since T 1 *a* gives the parent type T 1. The frequency with which T 1 *b* types are recovered from T 1 is 0·4 % and from T 1 *a*, 0·3 % indicating that the change to T 1 *b* occurs at the same rate in T 1 and T 1 *a*. The reversion from T 1 *a* to T 1 probably takes place at the same rate as the original change (T 1 to T 1 *a*), since T 1 gives 2·2 % of T 1 *a* types and T 1 *a* gives 1·7 % of T 1 type. T 1 a_2 appears to be more variable than T 1 and T 1 a_1 giving 18·9 % of variant colonies. T 1 a_2 sectors more frequently than the other types; it is probably, actually, as well as apparently, less stable than T 1 and T 1 *a*.

The question arises as to whether the changes are to be considered as being mutations or not. In so far as they are spontaneously occurring changes which are transmitted from cell to cell, they fall within the category of mutational changes. Since sexual reproduction has not been observed in this fungus it is not possible to follow the transmission of these changes during sexual reproduction. We cannot determine in this way whether the changes are transmitted by the nucleus. It is difficult however o see how results of such regularity could be obtained if the factors causing them are cytoplasmic. It may be possible to get some evidence regarding the location of these factors by studying diploid strains. 'Gigas' forms of a different strain of N.R.R.L. 1978B have been obtained by camphor treatment. These forms are probably diploid. It is hoped to obtain such 'gigas' forms of 1249 B21 and study variation in them. If the forms are diploid and if the changes which affect sporulating capacity occur in the nucleus, the variation pattern would be different from that of the haploid.

Mutation rate

It is clear that if the variants observed are due to mutations at a particular region (region being used in a general sense to mean either a gene or a piece of chromosome) the changes are of such a high frequency that the region may be considered a 'mutable region'. Such a mutability may be due to faulty reproduction of a particular segment leading to repeated reduplication and loss. If this is so, conditions affecting the rate of nuclear division might possibly affect the mutation rate. It is hoped to test the effect of environmental conditions such as changes of temperature on the rate of mutation in an attempt to obtain some clue as to the fundamental nature of the mutation.

The determination of the mutation rate is a very difficult problem. Delbrück (1945) has discussed the subject in relation to bacteria and much of his discussion is relevant to fungi also. So far as the radial growth of a colony on an agar surface is concerned it is clear that at any one point on the circumference of the colony there is very great competition as to which hyphae shall continue the growth and produce a new sector. When mutant nuclei are present in fewer numbers than the original nuclei, they have less chance of being present in the hyphae which carry on the growth of the colony. Mutants will only succeed in establishing themselves as sectors when they occur with a very high frequency or when they have an appreciably higher growth rate than the original type. So far as the aerial growth is concerned, the conditions have not been clearly worked out. However, it would be seen that if there is any weighting it would be in favour of the type present in greatest numbers, i.e. the original type. Therefore we may consider the percentage of mutant types recovered as a lower limit for the actual mutation rate. The actual mutation rate is *probably* not lower than the percentage of mutations recovered and is possibly higher.

Bunting (1940*a*, *b*) describes the occurrence of colour variation in *Serratia marcescens* which is similar in certain respects to the variation described in this paper. She found progressive variation in the sense that the dark red type gave a bright pink variant which in its turn gave a pale pink variant. She also found reversion since the bright pink and pale pink types both gave dark red types. This corresponds to the fact that T 1 *a* and T 1 a_2 both give **T** 1 types. However, in the case of *Serratia* the dark red types gave 2 % of bright pink colonies whereas the bright pink colonies gave 42 % of dark red colonies.

Skovsted (1943) has described what he calls 'successive mutation' in the yeast-like species, *Nadsonia Richteri*. Mutants were picked out as sectors reaching the edge in giant colonies. A number of single-celled cultures of the same original type each gave the same two types of mutants. Each of these types produced three new types. Two of these types were tested and each gave four new types. Five of these eight types were tested and gave altogether twenty-three new types. Thus although only two new types were obtained from the original type, many new types were derived from the mutants. Skovsted did not observe any examples of reverse mutation. However, the competition for survival in a colonial form is such that sectors can only be formed by mutants which occur very frequently and have a growth rate not less than the normal or by mutants which have a higher growth rate on the normal. The shape of the sectors illustrated by Skovsted shows that many of the mutants have a growth rate greater than that of the normal. In such conditions reverse mutations would not be normally detected even if they occurred because they could not compete with the new type. It might be possible, however, to detect them by altering the medium so as to favour the original type at the expense of the mutant. They could also be detected, if frequent, by plating out a large number of single cell colonies. Until such experiments have been made one cannot be sure that reverse mutations do not occur in *Nadsonia*.

Mycelial types

Mycelial types have been described for *Penicillium notatum* by Hansen and Snyder (1944). They relate the occurrence of such types to the 'dual phenomenon' which they have described in a large number of fungi imperfecti. Single spore (multinucleate) isolations of a number of fungi gave three types: *M*, mycelial, *C*, conidial and *MC*, intermediate. *M* types gave only *M* types, *C* types gave only *C* types, whereas *MC* types gave all three types when single spore isolations were made. *MC* types could be synthesized by putting *M* and *C* types together. *MC* types were thus found to be heterokaryons containing *M* and *C* nuclei. Later it was found that *M* types could arise from *C* types and that this often happens with great regularity whereas *M* types are stable, never having been observed to revert to *C* types although subjected to different cultural conditions. Earlier, Mohendra and Mitra (1930) described the occurrence of segregation of black colony (conidial) and white colony (mycelial) types during spore formation in *Sphaeropsis malorum*. This appears to be an example of the 'dual phenomenon' of Hansen (1938) and of Hansen and Snyder (1948) and here also the mycelial type did not revert to the conidial type. Mohendra and Mitra relate the supplantation of the black colony type by the white olony type during mass conidial transfers to the slower germination and educed viability of the black colony types.

Mycelial types have been observed mostly in fungi imperfecti and herefore it has not been possible to test the segregation of such types during xual reproduction. However, Hansen and Snyder (1943) were able to ross *M* and *C* strains of *Hypomyces Solani* f. *Curcurbitae*. It was found that the ycelial-conidial character segregated in a 1 : 1 ratio and was independent the incompatibility factors. It was also found that whereas the conidial pe was hermaphrodite the mycelial type did not produce perithecia and ould only function as a male. Hansen and Snyder therefore consider the hange from the conidial to the mycelial habit to be a true mutation from hermaphrodite to a male condition. In *Bombardia lunata*, Zickler (1937) ound the *lanata* factor, which appears to be a factor for mycelial growth, to e absolutely linked to a female 'sex realisator' and to be independent of he incompatibility factors. Robbins and Ma (1945) describe physiological xperiments on *Trichophyton mentagrophytes* in which they used the conidial ild type and a series of 'pleomorphic' types obtained from old cultures of he conidial type. The situation in *Trichophyton* resembles that in *Penicillium* n that a number of such types are found and in the non-reversion of these ypes to the conidial habit.

The mode of origin of these mycelial types is an interesting problem. The rthodox genetical hypothesis would be that they were mutations to a type r types which could grow on the surface of old cultures and which would end to be selected in transferring from old cultures. However, such ycelial types would not be expected to become established in the wild ecause of the absence of a satisfactory propagating mechanism such as hat provided by the conidia on the wild type. In *Hypomyces Solani* f. *ucurbitae* and *Bombardia lunata*, where the presence of a sexual stage permits he testing of the mutation theory, the mycelial type behaved as a single ene change, but in *Penicillium* and *Trichophyton* no such direct test is yet ossible. If the mycelial types are mutational in origin, the question arises s to whether the different mycelial types within one species are due to ifferent mutations or whether there is one mutation for mycelial types and he differences are due to modifying factors or cytoplasmic effects. The resence of the mutable series affecting sporulation capacity suggests that ycelial types may occur as an end result of this series. A frequently ccurring type of mutation such as this would account for the frequent ppearance of mycelial patches on old cultures. If this is so and all mycelial ypes are due to one type of change then such types when inoculated ogether would not give the wild type. This was reported by Pontecorvo and emmell (1944) in *Penicillium* and has been confirmed by me (1946). Also T 1 *a* nd T 1 a_2 when grown together do not give patches of T 1 type, which is in ccordance with the view that they are in some sort of allelic series. These esults however may be due to the less highly sporulating habit being dominant ver the conidial habit as suggested by Pontecorvo and Gemmell (1944).

It is hoped that further investigations will disclose whether there is a onnexion between the 'mutable region' and the production of mycelial ypes or not.

Penicillin production

In these experiments as in those of Raper and Alexander (1945) there was a negative correlation between sporulation capacity and penicillin production in the conidial types. This, however, may be due to some physiological or mechanical effect of sporulation on penicillin production rather than to a direct effect of the gene affecting sporulation on penicillin production. Mycelial types were found to be poor yielders.

A series of variants with increased production of yellow pigment showed a decreased production of penicillin. This accords with previous reports.

SUMMARY

The standard strain of 1249 B21 constantly throws paler and darker variants. The paler variants throw the darker variants, revert to the parental type and throw still paler variants. The latter are unstable giving all the darker types.

The colour is related to, and may be conditioned by, sporulating capacity.

The greater part of the variation exhibited by young cultures is due to changes in this particular 'mutable region'.

Patches of mycelial growth frequently occur on old single spore cultures as well as on old mass inoculation cultures. Mycelial types which do not normally revert to the conidial type can be isolated from these patches.

It is suggested that there may be a relationship between the 'mutable region' and the production of mycelial types but no direct link between the two phenomena has yet been observed.

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REFERENCES

Armitage, F. D. (1944). Chlorazol black E as a stain for mycological specimens. *Trans. Brit. myc. Soc.* xxvii, 131–3.

Bunting, M. I. (1940*a*). A quantitative study of the dark red to bright pink variation in *Serratia marcescens. J. Bact.* xxxix, 15.

Bunting, M. I. (1940*b*). A description of color variants produced by *Serratia marcescens. J. Bact.* xxxix, 108.

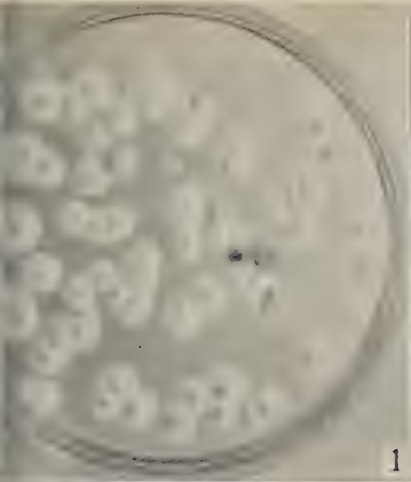
Delbrück, M. (1945). Spontaneous mutations in bacteria. *Ann. Mo. Bot. Gdn.* xxxi, 223–33.

Hansen, H. N. (1938). The dual phenomenon in imperfect fungi. *Mycologia*, xxx, 442–55.

Hansen, H. N. & Snyder, W. C. (1943). The dual phenomenon and sex in *Hypomyces solani* f. *cucurbitae. Amer. J. Bot.* xxx, 419–22.

Hansen, H. N. & Snyder, W. C. (1944). Relation of dual phenomenon in *Penicillium notatum* to penicillin production. *Science*, N.S. xcix, 2570, 264–5.

Mohendra, K. R. & Mitra, M. (1930). On the cultural behaviour of *Sphaeropsis malorum. Ann. Bot., Lond.*, xliv, 175, 541–55.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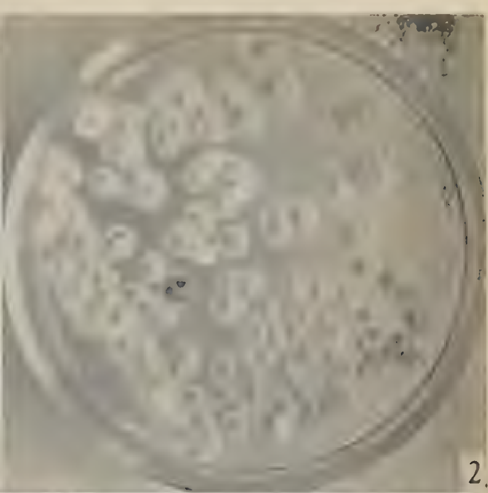
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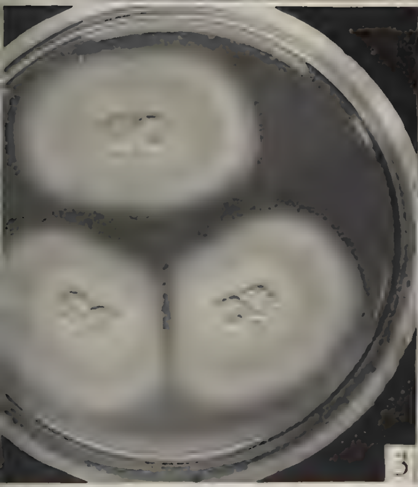
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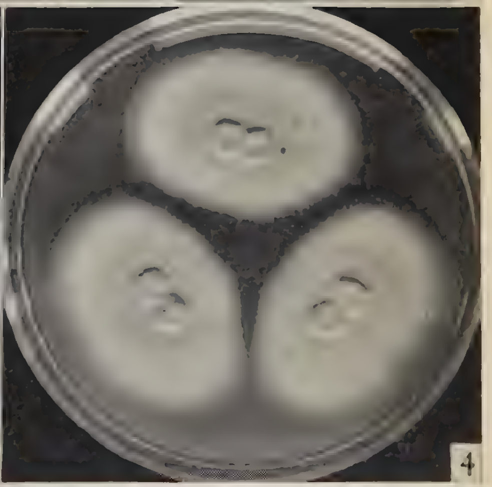
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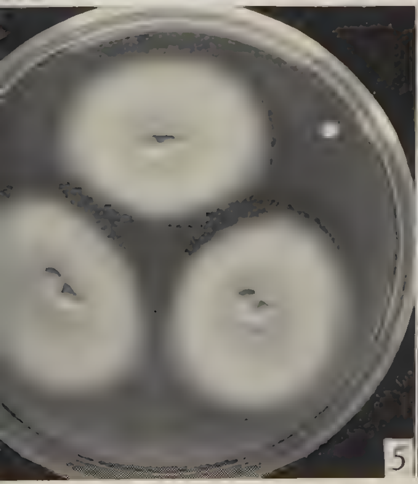
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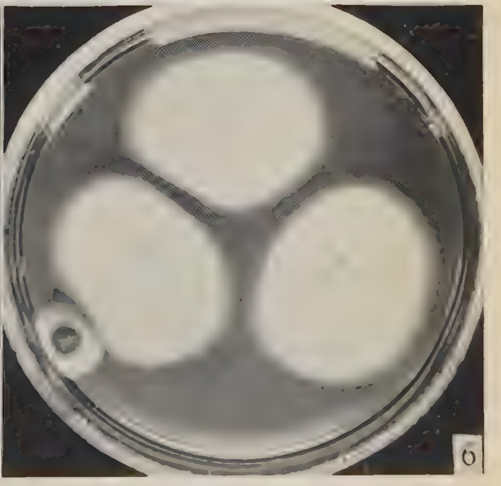
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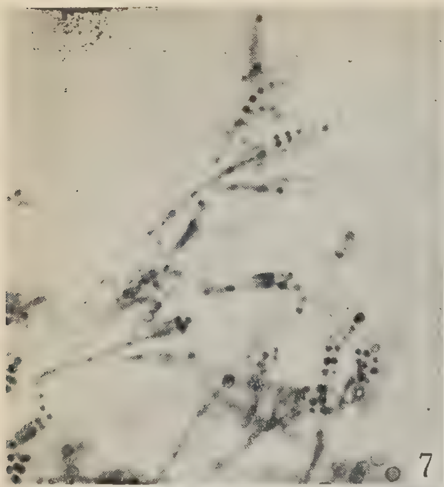
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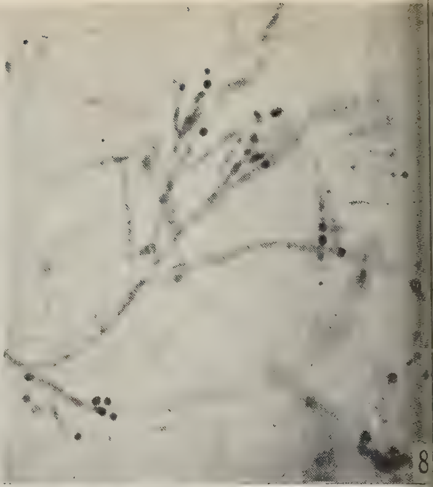
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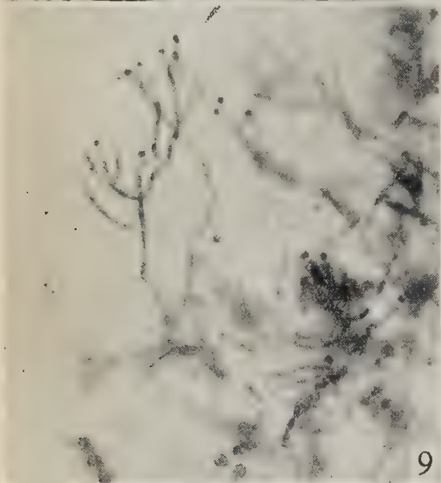
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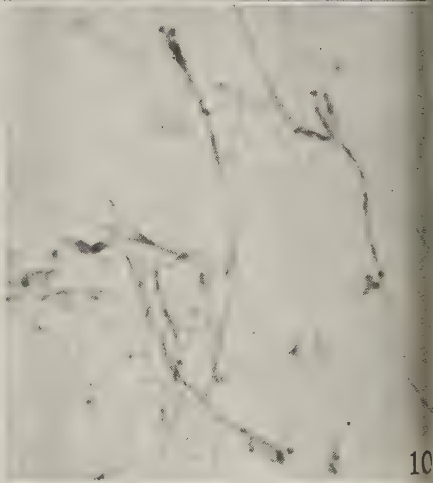
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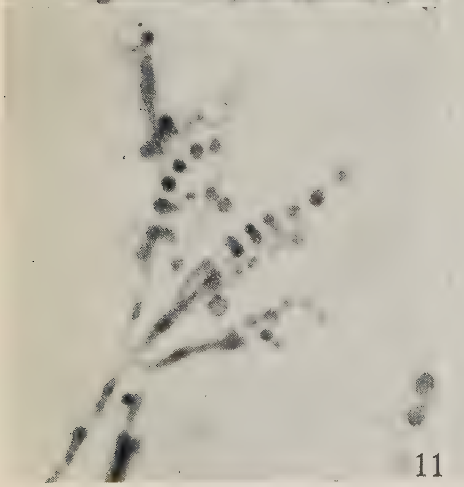
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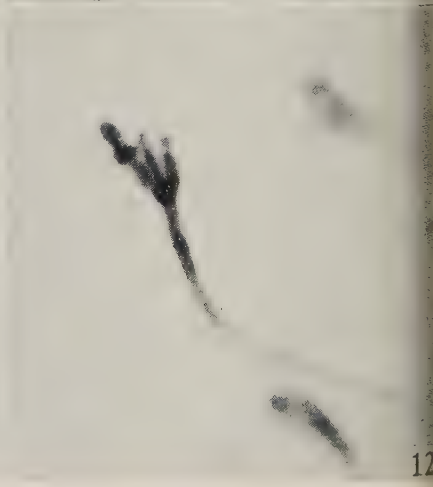
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ontecorvo, G. & Gemmell, A. R. (1944). Genetic proof of heterokaryosis in *Penicillium notatum*. *Nature, Lond.*, CLIV, 514–16.

aper, K. B. & Alexander, D. F. (1945). Penicillin. V. Mycological aspects of penicillin production. *J. Elisha Mitchell Sci. Soc.* LXI, 74–113.

obbins, Wm. J. & Ma (Roberta) (1945). Growth factors for *Trichophyton mentagrophytes*. *Amer. J. Bot.* XXXII, 509–23.

ansome, E. R. (1946). Induction of 'gigas' forms of *Penicillium notatum* by treatment with camphor vapour. *Nature, Lond.*, CLVII, 843.

kovsted, A. (1943). Successive mutations in *Nadsonia Richteri* Kostka. *C.R. Lab. Carlsberg*, Série physiol. XXIII, 409–53.

ickler, H. (1937). Die vererbung des Geslechts bei dem Ascomyzeten *Bombardia lunata* Zckl. *Z. indukt. Abstamm.- u. VererbLehre*, LXXVIII, 403–8.

EXPLANATION OF PLATES

Plate VI

All cultures photographed on malt agar

g. 1. Seven-day colonies from a plating of T 1 *a* type. Three variants of T 1 type and two of T 1 *b* type are visible.

g. 2. Seven-day colonies from a plating of T 1 type. One variant of T 1 *b* type and eight of T 1 *a* type are visible (dark colonies on right due to shadow).

g. 3. Inoculation of one T 1 *b* strain at top of Petri dish, inoculation of a second T 1 *b* strain of independent origin at left of Petri dish and side-by-side inoculations of two strains at the right of the Petri dish. One strain shows a mutant sector.

g. 4. Similar inoculations of two strains of T 1 type separately and combined (combination at the right).

g. 5. Similar inoculations of two strains of T 1 *a* type.

g. 6. Similar inoculations of two strains of T 1 a_2 type.

gs. 3–6. Eight-day cultures.

Plate VII

Photomicrographs, figs. 7–10 × 500; figs. 11, 12 × 1000

g. 7. T 1 *b* from nine-day culture.

g. 8. T 1 from nine-day culture.

g. 9. T 1 *a* from nine-day culture.

g. 10. T 1 a_2 from twenty-six-day culture.

g. 11. Single penicillus of T 1 *b*.

g. 12. Single penicillus of T 1 a_2.

Slides fixed formol alcohol, stained chlorazol black and mounted gum chloral.

(*Accepted for publication* 2 *November* 1946)

ON *MYZOCYTIUM MEGASTOMUM* DE WILDEMAN

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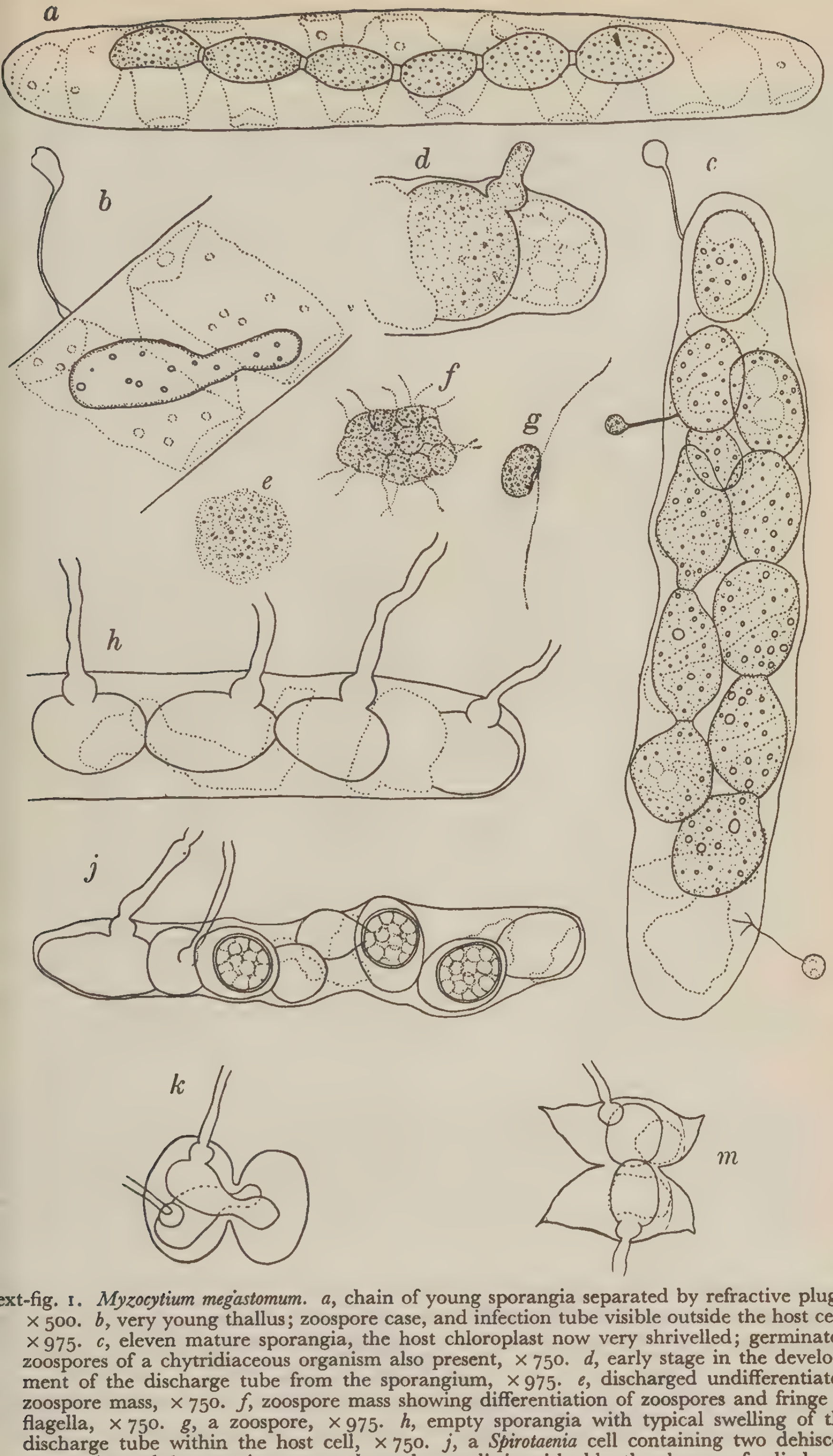
(With Plate VIII and 3 Text-figures)

Since *Myzocytium megastomum* was originally described by De Wildeman (1893) a number of references to it have appeared in the literature (Scherffel, 1914; Skvortzow, 1925; Cejp, 1935; Berdan, 1938; Karling, 1942), but because of the lack of knowledge of the zoospores, its exact systematic position has remained in doubt. According to Sparrow (1943), this species has not been recorded from Britain. However, Prof. C. T. Ingold (personal communication) in 1944 found this organism attacking *Euastrum ansatum*, *Closterium costatum*, *C. rostratum* and *Pleurotaenium Ehrenbergii* from Woodhouse Eaves near Leicester (Text-fig. 2 *a–f*), but again the zoospores were not observed. In July 1946 I found *Myzocytium megastomum* parasitizing *Spirotaenia condensata* Brèb., in Little Green Tarn, Claife Heights, near Hawkeshead, Lancashire, and the biflagellate zoospores were observed, showing that this fungus really belongs to the genus *Myzocytium*. A single specimen of what is believed to be this fungus was found in *Closterium* from the Clay Pond, Wray Castle, Windermere, in September 1946. It differs from the material from Green Tarn and Woodhouse Eaves in the more globular zoosporangia (21–26 μ in diameter) and in the larger zoospores (9 μ in diameter). Mature sporangia and stages in the formation of the biflagellate zoospores are shown in Text-fig. 3, and Pl. VIII, figs. 3, 4. Young thalli and resting spores were not observed. An account of *Myzocytium megastomum* on *Spirotaenia condensata* follows.

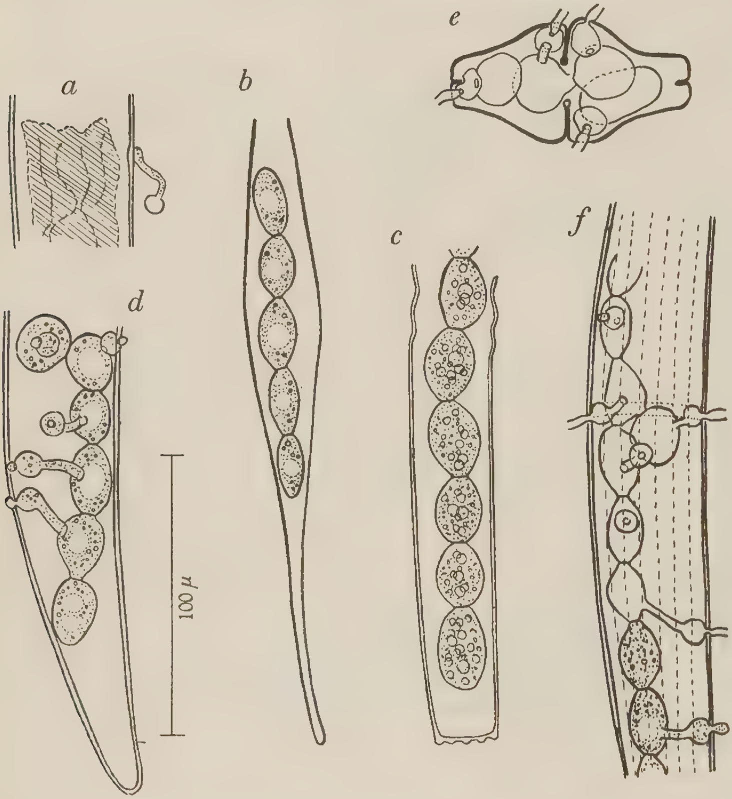
The young endobiotic thallus is relatively short and not subdivided by constrictions. At this early stage of development the empty encysted zoospore and its germ tube apparently connected with the thallus, could usually still be seen outside the host cell (Text-fig. 1 *b*). The thallus elongates and becomes constricted into portions each of which later becomes a single sporangium. The young sporangia are often separated from one another by a plug of refractive material (Text-fig. 1 *a*) which disappears as the sporangial wall thickens. Such plugs were not observed by Prof. Ingold in the material from Woodhouse Eaves (Text-fig. 2 *b*, *c*). The number of sporangia from any one infection into which the thallus is transformed varies from about one to eight, but more than one thallus may be found in a single host cell. The mature sporangia are ovoid, ellipsoidal or spherical (32–19·8 μ long × 15–13·2 μ diam.), with hyaline cytoplasm containing many scattered highly refractive globules (Text-fig. 1 *c*).

By the time the sporangia are mature the host chloroplast is reduced to a brown residue, although it still exhibits a somewhat spiral form. At last, from each sporangium a discharge tube is formed which is expanded



ext-fig. 1. *Myzocytium megastomum*. *a*, chain of young sporangia separated by refractive plugs, × 500. *b*, very young thallus; zoospore case, and infection tube visible outside the host cell, × 975. *c*, eleven mature sporangia, the host chloroplast now very shrivelled; germinated zoospores of a chytridiaceous organism also present, × 750. *d*, early stage in the development of the discharge tube from the sporangium, × 975. *e*, discharged undifferentiated zoospore mass, × 750. *f*, zoospore mass showing differentiation of zoospores and fringe of flagella, × 750. *g*, a zoospore, × 975. *h*, empty sporangia with typical swelling of the discharge tube within the host cell, × 750. *j*, a *Spirotaenia* cell containing two dehisced sporangia, and three resting spores; the males are distinguished by the absence of a discharge tube, × 500. *k*, *Cosmarium contractum* with possible dwarf thalli, × 500. *m*, the same in *Staurastrum lunatum*, × 500.

immediately within the host wall. This is the characteristic feature of this species. According to the place of germination of the sporangia relative to the host cell wall, the endobiotic swelling may either be almost sessile on the sporangium (Text-fig. 1 *d*, *h*) or at some distance from it (Text-fig. 2 *d*, *f*). The discharge tube, except for the intramatrical swelling (4–6 μ in diameter), is equally cylindrical throughout its length (2·6 μ wide) and may extend up to 30 μ in length outside the host wall (Text-fig. 1 *h*). The extramatrical prolongation of the discharge tube in the material from Woodhouse Eaves appears to be relatively short (Text-fig. 2 *e*, *f*). On deliquescence of the apex of the discharge tube, the contents of the sporangium emerge to form a spherical granular mass 17 μ in diameter with numerous small refractive globules (Text-fig. 1 *e*). This mass undergoes slight amoeboid movements and, some ten minutes later, the zoospores



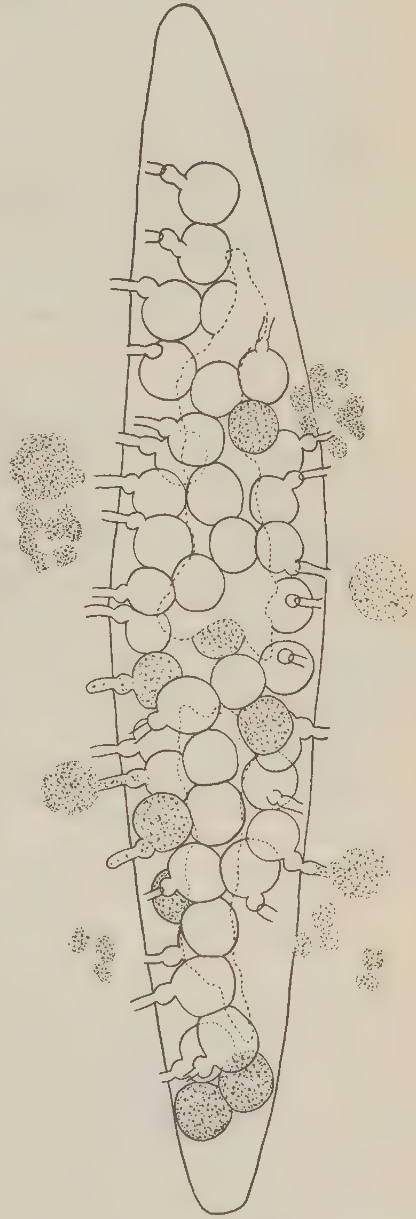
Text-fig. 2. *Myzocytium megastomum*. *a*, empty zoospore case with germ tube on *Closterium costatum*; *b*, chain of sporangia each with a conspicuous vacuole in *Closterium rostratum*; *c*, chain of sporangia in *Pleurotaenium Ehrenbergii*; *d*, germinating sporangia, each discharge tube swollen immediately inside the algal wall, in *Closterium costatum*; *e*, four empty sporangia in *Euastrum ansatum*; *f*, chain of sporangia in various stages of development in *Closterium costatum*. In most of the figures the disintegrating host contents are omitted. (Drawn by C. T. Ingold.)

adually become differentiated, and the flagella appear as a fringe of
ort, actively waving structures around its periphery (Text-fig. 1 *f*). The
ospores remain entangled by their flagella for some time, but finally
eak free and swim away individually.
ach zoospore mass becomes resolved
to ten to fourteen somewhat bean-
aped zoospores (4·5–5 μ long × 5·6–
μ diam.) with granular protoplasm
ntaining several small refractive
obules. There are two flagella of
out equal length inserted laterally
a slight depression. One flagellum
directed backwards and the other
rwards when swimming (Text-fig.
g).

Resting spores are formed abun-
ntly together with the sporangia,
d agree with those described by De
ildeman (1893). The mature resting
ores are spherical, 13–15 μ in di-
neter, with a smooth thick wall, and
ly contents (Text-fig. 1 *j*). No con-
ncing young stages in the develop-
ent of these resting spores were seen,
t it is clear that they are formed by
sexual process. In only one specimen
as any definite fertilization tube con-
ecting the male and female game-
ngia observed (Text-fig. 1 *j*). The
ale gametangia can always be dis-
nguished from the empty sporangia
the lack of discharge tubes. The
sting spores are usually formed at
e side of the female gametangium
earest the male. Their germination
as not observed.

Young thalli of a chytridiaceous
ganism were also present on the
irotaenia cells (Text-fig. 1 *c*), but
rther stages in the life history of this
ngus are not yet known.

The following records of *Myzocytium*
egastomum have already been made:
om Belgium, Switzerland and
orway (De Wildeman, 1893, 1895,
96); Hungary (Scherffel, 1914);
anchuria (Skvortzow, 1925); Bo-
mia (Cejp, 1935); and America



Text-fig. 3. *Myzocytium megastomum*. Sporangia in various stages of development in *Closterium* sp. × 300.

(Berdan, 1938). Karling (1942) rightly lists *Ancylistes miurii* Skvortzow (1925, p. 432, figs. 7–10) as a synonym.

From the many observations on *Myzocytium megastomum* it would seem that the expansion of the discharge tube within the host wall is a good specific distinction separating *M. megastomum* from the closely allied *M. proliferum* (Schenk). If this is so, then, the *M. proliferum* of Martin (1927; in *Cladophora* sp.), the specimen recorded by Sparrow (1943; in *Closterium costatum*, Farlow Herbarium no. 642), and that of De Wildeman (1895, p. 76, pl. 2, figs. 7–9; in *Euastrum*) should be included in *Myzocytium megastomum*, a course not followed by Karling (1942). De Wildeman's specimen in *Euastrum* is peculiar in producing only one sporangium instead of a chain of sporangia, and probably represents a reduced form. Similar simplified thalli were seen in the Leicestershire collection (Text-fig. 2*e*); by myself in August 1946 from the plankton of Lake Windermere, South Basin, in *Cosmarium contractum* (Text-fig. 1*k*) and *Staurastrum lunatum* (Text-fig. 1*m*), and by Petersen under *Myzocytium irregulare* (1909; 402, fig. 16*d*; 1910; 538). Karling (1942), in agreement with Fischer (1892), De Wildeman (1896) and Minden (1911), has suggested that *Bicrium transversum* and *Bicrium naso* (Sorokin, 1883) may also represent dwarf thalli of *Myzocytium*, and since *B. naso* has an endobiotic swelling on the discharge tube this species would be referable to *M. megastomum*. The superficial similarity of these simplified forms of *M. megastomum*, in the smaller desmids, with *Olpidium immersum* Sorokin cannot be overlooked, and since the zoospores of *O. immersum* have not been observed this may be found to belong to the genus *Myzocytium*. However, only when the zoospores, and resting spores of dwarf thalli of *Myzocytium*, *Bicrium naso* and *Olpidium immersum* have been observed, and inoculation experiments on various desmids been carried out, will the true nature of these fungi be established.

My thanks are due to the Director of The Freshwater Biological Association, Wray Castle, Windermere, for the use of a laboratory in which this work was done, and especially to Prof. C. T. Ingold for helpful criticism, and permission to publish his figures of *Myzocytium megastomum*.

REFERENCES

BERDAN, H. (1938). Revision of the genus *Ancylistes*. *Mycologia*, XXX, 396–415.

CEJP, K. (1935). The parasites of Conjugates in Bohemia. IIIrd contribution. *Bull. Int. Acad. Sci. Bohème*, XLV, 1–12. (Separate.)

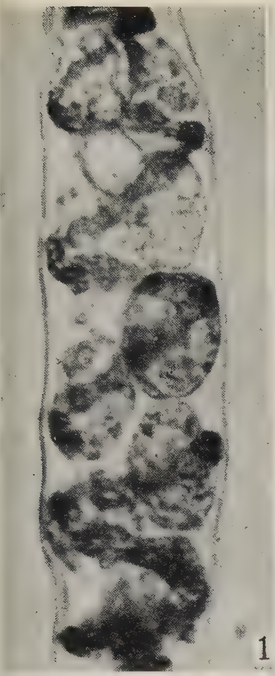
FISCHER, A. (1892). Phycomycetes. Die Pilze Deutschlands, Oesterreichs und der Schweiz. *Rabenhorst. Kryptogamen-Fl.* I (4), 1–490. Leipzig.

KARLING, J. (1942). *The simple holocarpic biflagellate Phycomycetes.* New York City.

MARTIN, G. W. (1927). Two unusual water moulds belonging to the family Lagenidiaceae. *Mycologia*, XIX, 188–90.

MINDEN, M. VON (1911). Chytridiineae, Ancylistineae, Monoblepharidineae, Saprolegniineae. *Kryptogamenfl. Mark Brandenburg*, V, pt. 3, pp. 353–496.

PETERSEN, H. E. (1909). Studier over Ferskvands-Phycomyceten. Bidrag til Kundskaben om de submerse Phycomyceters Biologi og Systematik, samt om deres Udbredelse i Danmark. *Bot. Tidsskr.* XXIX (4), 345–440.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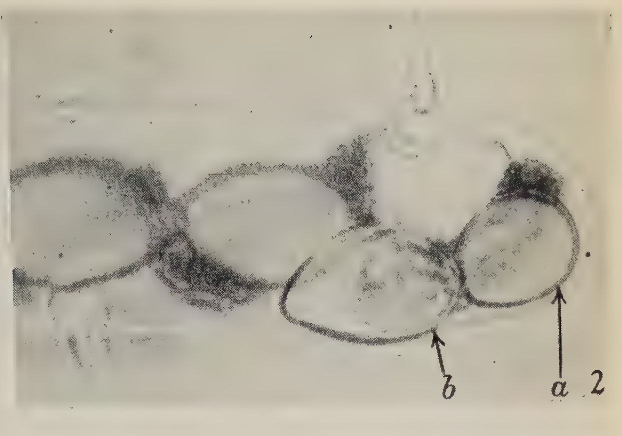

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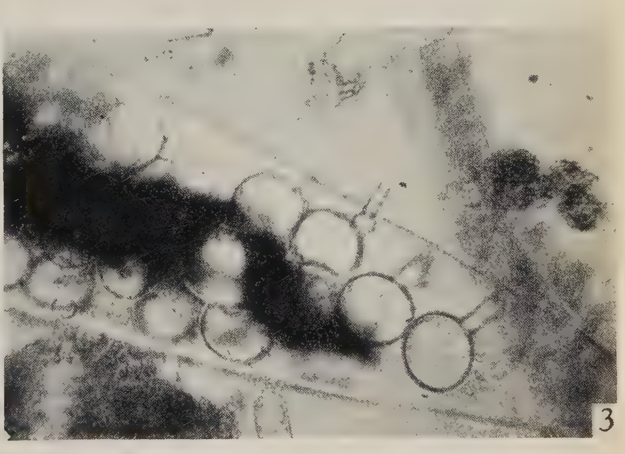
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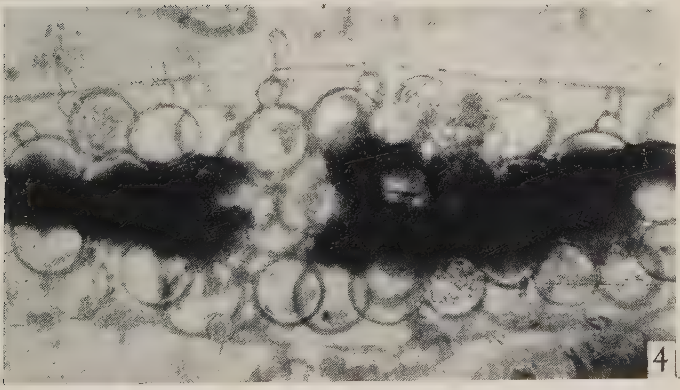
4

Petersen, H. E. (1910). An account of Danish freshwater Phycomycetes, with biological and systematical remarks. *Ann. Mycol., Berl.*, viii, 494–560.

Scherffel, A. (1914). Kisebb Közlemények a kryptogamok köréböl (Kryptogamic Miszellen). *Bot. Közl.* xiii, 12–17.

Skvortzow, B. W. (1925). Zur Kenntnis der Phycomyceten aus der Normandshurei, China. *Arch. Protistenk.* li, 428–33.

Sorokin, N. W. (1883). Aperçu systématique des Chytridiacées recoltées en Russie et dans l'Asie Centrale. *Arch. Bot. Nord. Fr.* ii, 1–42. (Issued as a separate.)

Sparrow, F. K. (1943). *Aquatic Phycomycetes.* Ann Arbor, U.S.A. University of Michigan Press.

Wildeman, É. De (1893). Notes mycologiques. II. *Ann. Soc. Belge Micro.* (*Mém.*) xvii, 35–63.

Wildeman, É. De (1895). Notes mycologiques. IV. *Ann. Soc. Belge Micro.* (*Mém.*) xix, 59–80.

Wildeman, É. De (1896). Notes mycologiques. VII. *Ann. Soc. Belge Micro.* (*Mém.*) xx, 21–64.

EXPLANATION OF PLATE VIII

Myzocytium megastomum De Wildeman

Fig. 1. Part of a *Spirotaenia* cell containing sporangia. The disorganized spiral chloroplast the host is clearly visible. × 780.

Fig. 2. Three empty zoosporangia and a resting spore in *Spirotaenia*: (*a*) the male, (*b*) the female, containing a thick-walled resting spore. × 1230.

Figs. 3, 4. Parts of a *Closterium* cell from the Clay Pond, Wray Castle, with zoosporangia in various stages of development. The swelling of the discharge tube immediately within the host wall is well marked. × 450.

(*Accepted for publication* 5 *November* 1946)

BRITISH FUNGI

By W. C. MOORE, *Plant Pathology Laboratory, Harpenden*

1. *Peronospora Dipsaci* Tul., in *C.R. Acad. Sci., Paris*, xxxviii, 1103 (1854); Gäumann, *Monogr. Gattung Peronospora*, 238 (1923).

On leaves of *Dipsacus sylvestris* L., Dundry, Somerset, May 1941 (L. Ogilvie) and Bricketwood, Herts, October 1946 (A. Smith).

2. *Sphaerotheca fuliginea* (Schlecht.) Salm., in *Mem. Torrey bot. Cl.* xxix (1902).

On cultivated *Doronicum*, Wye, Kent, July 1938 (W. M. Ware); Luton, Bedfordshire, October 1939. Probably widely distributed.

In the material from Luton the perithecia were 81–96 μ (average 87 μ) in diameter, the asci 62–72 × 51–55 μ (average 67 × 52 μ) with 6–8 ascospores.

3. *Oidium* sp.

On *Antirrhinum majus* L., Bucks, March 1928 (W. Buddin); Devon, Newton Abbot, April 1928 (A. Beaumont); Cambridge, February 1934 (F. T. Brooks); and later at Cardiff (J. Rees); Shrewsbury (N. C. Preston) and Harpenden.

Usually slight, under glass, and probably not uncommon. At Harpenden seen also after planting out, and at Shrewsbury severe on bedded out plants. At Harpenden forming a sparse white web or dusty covering on undersurfaces of leaves, and occasionally on upper surface. Conidia hyaline, almost rectangular, with slightly rounded ends or occasionally barrel-shaped, 24–45 × 12–17 μ (average of forty spores 30 × 14·5 μ).

4. *Oidium* sp.

On soft young tips of gorse shoots (*Ulex* sp.) in the Malvern Hills, July 1931 (G. H. Pethybridge).

The only previous record of a powdery mildew on *Ulex* appears to be one in Ireland in 1906. Adams (1906) recorded *Cicinnobolus Ulicis* n.sp. as 'parasitic on one of the Erysiphaceae on stems and leaves of *Ulex europaeus*, which it covers with a felt of hyphae bearing pycnidia (Figs. 2, 3 and 4), July. On Great Sugar Loaf, Co. Wicklow.' A specimen of the Irish material from the Crypt. Coll. Dublin, when examined in 1931, showed very little mildew and that only in the *Oidium* stage.

5. *Gibberella moricola* (de Not.) Sacc., *Michelia*, i, 347 (1879); Sacc. *Syll. Fung.* ii, 553.

On dead fig twigs (*Ficus Carica* L.), Dorset, January 1946 (W. Buddin).

Perithecia present in large numbers, mostly immature though a number of ripe ones found, free on substratum or partly immersed, scattered or aggregated into small tightly packed groups, black (blue in transmitted light), 210–450 μ (mostly 240–300 μ) in diameter, and mixed here and there with a few immature buff-coloured ones. Asci cylindrical or club-shaped, 60–90 μ long, containing eight monostichous or subdistichous ascospores, sometimes fewer. Ascospores straight or occasionally slightly curved, variable in size and shape, usually egg-shaped to spindle-shaped, but sometimes broader at one end than the other, 1–5, mostly 3-septate, generally with a slight constriction at the septum, 16–25 × 6–9 μ (average of twenty spores 20 × 7 μ), those with two septa broad in relation to their length. A few orange-pink sporodochia of a species of *Fusarium* present among the perithecia, with immature sickle-shaped spores, 20–31 × 3–4 μ, having acute ends and no discernible septa.

Wollenweber and Reinking (1935) distinguished *Gibberella baccata* (Wallr.) Sacc. var. *moricola* (de Not.) Wollenw. (= *G. moricola*) from *G. baccata* (Wallr.) Sacc. by its somewhat smaller spores. Miss E. M. Wakefield has, however, drawn my attention to the fact that in *Syll. Fung.* II, 553, Saccardo gives the larger spores (24–30 × 6–7 μ) for *moricola* and smaller spores (16 × 8 μ) for *baccata*. But when Miss Wakefield examined two of Saccardo's specimens in Herb. Kewensis, which had been distributed in Myc. Ven. nos. 652 and 653 as *Botryosphaeria pulicaris* f. *Mori albae* on *Morus alba*, and *B. pulicaris* f. *baccata* on *Robinia pseudacacia*, respectively, she found that the relative spore sizes were the reverse of those given in the *Sylloge*. The form on *Morus* had spores which were straight and rather cylindric, narrowing only comparatively slightly towards the ends; and measuring 16–22 × 5–6 μ. The *baccata* form had larger, more fusiform spores (25–30 × 6–7 μ), most of which were slightly curved. From my observations, the material on *Ficus* from Dorset corresponded more closely to the form on *Morus* than to the *baccata* form in Saccardo's specimens, and it is therefore identified with *Gibberella moricola*.

The record of *G. moricola* on *Morus* in Hants, listed by Bisby and Mason (1940), is an error of transcription: the imperfect stage only of the fungus (*Fusarium lateritium* Nees) was reported in the paper cited.

6. *Leptosphaeria heterospora* (de Not.) Niessl, *Beitr.* 23 (1872); Sacc. *Syll. Fung.* II, 67.

On rhizomes and roots of *Iris germanica* near Woking, Surrey, March 1930; on var. Ann Page in St James's Park, London, October 1930; on *I. pumila formosa* in Kent 1932; and on *I. germanica* near Aylesbury, Buckinghamshire in May 1946.

The fungus is found on unthrifty and yellowing plants and is probably a weak parasite. After making healthy but rather weak growth for several weeks in spring, the outer foliage becomes brown and withered, and the younger leaves turn yellow and begin to die back from the tips. On lifting affected plants, the rhizomes appear firm and show no decay, but most of the roots are withered and dead, or are reduced to discoloured, hollow tubes. The black perithecia can be found in appreciable numbers partially embedded in the surface of the rhizomes, especially on the underside, and there are usually a few on the roots. The *Leptosphaeria* was associated with a species of *Phoma* (with spores 4–6 × 1·5–2 μ) in the material from Aylesbury, and with *Septoria* sp. in that from Kent.

7. *Ophiobolus rubellus* (Pers. ex Fr.) Sacc. *Michelia*, II, 324 (1882); Bisby and Mason in *Trans. Brit. myc. Soc.* XXIV, 194 (1940); syn. *O. porphyrogonus* (Tode) Sacc. *Syll. Fung.* II, 338.

On roots of unpulled and badly weathered flax (*Linum usitatissimum* L.), Pembrokeshire (Hayscastle and Dinas Cross), September 1945 (D. L. G. Davies).

8. *Phyllosticta camelliaecola* Brun. var. *meranensis* Bubák, in *Öst. bot. Z.* 80 (1905); Sacc. *Syll. Fung.* XVIII, 224 (1906).

On living leaves of *Camellia*, St Albans, Herts, May 1939. Spots rounded, brown or paling, with a well-defined darker, narrow margin, raised especially on the underside, and believed to be caused primarily by insects. Pycnidia on both sides of spots, scattered, small, immersed. The spores in this specimen agreed with those of Bubák's variety (3–5 × 1–1·5 μ).

Phyllosticta camelliaecola Brun. *Misc. Mycol.* p. 13; Sacc. *Syll. Fung.* X, 101 (1892), with spores 5–6 × 2–3 μ, is regarded by Grove, *Coelomycetes*, I, 10 (1935), to be the same as *P. Camelliae* Westd. in Kickx, *Flor. Crypt.* I, 416, for which there are no spore measurements. *P. Camelliae* was recorded from Cound, Salop, in January 1939 (N. C. Preston), but no specimen is available.

9. *Phomopsis viticola* Sacc., in *Ann. Mycol., Berl.*, XIII, 118 (1915); Grove, *Coelomycetes*, I, 237 (1935).

On base of shoot of a vine branch, Dunraven Castle, Glamorgan, May 1931 (J. Rees).

10. *Sphaeronema pruinosum* Peck in Rep. St. Mus.

On branch of a six-year-old apple tree at Otley (Yorks), April 1928; the branch was infested with Mussel Scale and was rather extensively cankered.

Pycnidia almost superficial, more or less conical or columnar, containing macro- and microspores. The larger spores measured 16–27 × 9–12 μ (average of twenty, 23 × 10 μ) and were mixed with large numbers of hyaline, straight or slightly curved, unicellular, filiform spores, 6–13 × 1–2 μ (average length of twenty, 9·3 μ). The characters of the fungus agreed closely with those of *Glutinium macrosporum* Zeller, described by Zeller (1927) as the cause of a canker of apple and pear trees in Oregon. Examination of some of the Oregon material, however, which Dr Zeller sent at my request, revealed certain minor differences between the two fungi, which were not regarded as sufficient to justify specific distinction. According to Zeller (1927) the pycnidia of *G. macrosporum* produced in culture had smaller spores than those (15–28 × 8–10·5 μ) found in nature. The spores were hyaline and showed only false septation on germination. The fungus induced a slow rot of apple fruits, but no pycnidia developed on the fruit. The spores of the English fungus in culture were up to 30 μ long. Moreover, some of the spores became pale brown, and on germination they sometimes became truly 1–3-septate. Pycnidia similar to those in culture developed on apple fruits slowly rotted by the fungus.

Miss E. M. Wakefield, who also examined the two fungi, pointed out that *G. macrosporum* Zeller did not differ from *Sphaeronema pruinosum* Peck, the conidial stage of *Pezicula pruinosa* Farlow, which was originally described on *Amelanchier*. She examined material in Herb. Kewensis on *Amelanchier* from Farlow's Herbarium, and also a specimen collected by J. B. Ellis, said to be on elder, which Peck had identified as *Sphaeronema pruinosum*, and they did not differ from one another or from *Glutinium macrosporum*. Later, Zeller (1935), having examined Peck's type, accepted this view, though he did not regard the fungus as a good species of *Sphaeronema*.

11. *Ascochyta bohemica* Kab. & Bub., in *Hedwigia*, XLIV (1905); Sacc. *Syll. Fung.* XXII, 1024 (1913).

On leaves of *Campanula medium* L., Harpenden, September 1941 (J. M. Gooby). Previously found in Britain on *C. betulaefolia* and *C. Ranieri* at Maidenhead (*Trans. Brit. myc. Soc.* XXIV, 60).

On *C. medium* the spots were round or sub-angular, 2–10 mm. in diameter, epiphyllous at first, later visible on both sides of leaves, brown, dry, with a broad purple margin 2 mm. wide. Pycnidia few, with spores mainly unicellular and biguttulate, mostly $12 \times 6\,\mu$, occasionally 1-septate, constricted at the septum and then with several guttules. Perhaps an immature *Stagonospora*.

12. *Ascochyta Cinerariae* Tassi, in *Boll. Orto. bot. Siena*, 31 (1899); Sacc. *Syll. Fung.* XVI, 930.

On decayed stem bases of cineraria plants in pots, at Carrington and Manchester, Lancashire, December 1934 (E. Holmes Smith); and at Woburn Sands, February 1940 (W. Buddin).

At Woburn Sands associated with a basal stem rot causing heavy loss among plants growing in fresh loam in new pots. Pycnidia numerous, aggregated, erumpent, globose or lens-shaped, very variable in size, 180–250 μ (–300 μ) in longer axis (average of ten, 200 μ), pale brown, thin-walled parenchymatous, cells somewhat thickened and darker around a well-defined ostiole 25–35 μ in diameter. Spores hyaline or slightly coloured, egg shaped, $6\text{–}10 \times 3\text{–}5\,\mu$, mostly 1-septate when mature. In Lancashire associated with basal stem rot in 40 % of young pot plants. This fungus agrees closely with *Ascochyta Cinerariae* Tassi, which has been described as the cause of a leaf spot of cinerarias in Germany (Wasewitz, 1936) and Italy. The pycnidia are lighter than those of *A.* (*Diplodina*) *Lycopersici*, from which the fungus is otherwise not dissimilar. *Diplodina* (*Ascochyta*) *fibricola* (Sacc.), recorded on rotting stems of *Cineraria maritima* in France (Rab. *Krypt. Fl.* I, vi, 682 (1901)), appears to be different.

It is uncertain whether *Ascochyta Cinerariae* is parasitic on the stem bases of cinerarias, producing a disease similar to but distinct from Foot Rot caused by *Phytophthora cryptogea* Pethybr. & Laff., or whether it merely develops secondarily on the stem bases of plants primarily affected with Foot Rot. No *Phytophthora* was found in the Woburn material.

13. *Ascochyta dahliicola* (Brun.) Petr., in *Ann. Mycol. Berl.*, xxv, 201 (1927); syn. *Phyllosticta dahliicola* Brun., *Champ. Saint.* 429 (1887); Sacc. *Syll. Fung.* x, 129 (1892); Grove, *Coelomycetes*, I, 14 (1935).

On *Dahlia variabilis* Desf., Wokingham, Berks, September 1937 (W. Buddin); var. Clara Carder, Harpenden, Herts, October 1941 (W. Buck).

14. *Ascochyta Impatientis* Bres., in *Hedwigia*, 326 (1900); Sacc. *Syll. Fung.* xvi, 927.

On living leaves of *Impatiens balsamina* L., Newton Abbot, Devon, October 1942 (A. Beaumont).

Pycnidia scattered or aggregated, globose or flattened, immersed, thin-walled, pale brown and translucent, 120–150 μ diam., opening with a pore about 15 μ wide. Spores issuing in dense clouds, straight cylindrical with rounded ends, 6–11 × 2–4 μ, mostly 9 × 3 μ, ultimately 1-septate, not constricted at the septum.

A. Weissiana Allesch., Rab. *Krypt. Fl.* I, vi, 647 (1899); Sacc. *Syll. Fung.* xvi, 927, on *Impatiens balsamina* in Saxony, may be the same but is described as having black-brown pycnidia, and spores measuring 10–16 × 3–4·5 μ.

15. *Botryosporium longibrachiatum* (Oudem.) Maire, in *Ann. Mycol. Berl.*, I, 340 (1903); Mason in *Annotated Account of Fungi received at the Imperial Bureau of Mycology*, List II (Fascicle 1), 27.

On tomato stems, Rustington, Sussex, September 1943 (W. A. Millard).

16. *Cercosporella Primulae* Allesch., in *Ber. Bayr. Bot. Ges.* II, 18 (1892); *Hedwigia*, xxiv, 286 (1895); Sacc. *Syll. Fung.* xi, 607.

On living leaves of *Primula Wanda*, Staffs, July 1928; and on hybrids of *P. Juliae*, Bridge Sellers, Hereford, August 1931, Gamons, Hereford, March 1933 (L. Ogilvie), and Bartley, Southampton, May 1936 (*Trans. Brit. myc. Soc.* xxv, 208).

17. *Fusicladium Lini* Sorauer, in *Z. PflKrankh.* v, 103 (1895); De Wild. & Dur. *Prodr. Fl. Belg.* II, 336.

Forming a black coating on wilted cotyledons and lower leaves of *Linum usitatissimum* L., Egmere, Norfolk (R. E. Taylor) and in Wiltshire (L. Ogilvie), June 1942.

Conidiophores simple, usually fasciculate, septate, pale brown, more or less rigid, straight or somewhat bent, ultimately up to over 200 μ long, and 3–6 μ broad. Spores borne singly at tips and at times apparently pleurogenous by the continued growth of the conidiophore, mostly straight, or sausage-shaped, pale coloured, with a clearly defined wall about 1 μ thick, minutely apiculate, 12–37 × 5–9 μ (average of twenty-five spores 24 × 7 μ), continuous or 1-septate, sub-catenulate. Sorauer did not give a full description of his fungus and gave the spores as only 8 × 4 μ, with some 14–16 μ long.

Sorauer regarded *Fusicladium Lini* as a parasite and the cause of wilting and discoloration of the upper portions of flax seedlings in Belgium, van Poeteren (1929) found what he thought was the same species, or possibly only *Cladosporium herbarum*, on yellow and dwarfed flax seedlings in Holland. In England the fungus was associated at both localities with yellowing and slow wilting of young seedlings and of plants just beginning to flower.

I am very much indebted to Miss E. M. Wakefield for continued help and interest and to all those collaborators who have sent me material.

REFERENCES

ADAMS, J. (1906). Irish parasitic fungi. *Irish Nat.* XVI, 168–9.
BISBY, G. R. & MASON, E. W. (1940). List of Pyrenomycetes recorded for Britain. *Trans. Brit. myc. Soc.* XXIV, 197.
POETEREN, N. VAN (1929). Verslag over de werkzaamheden van den Plantenziektenkundigen Dienst in het jaar 1928. *Versl. PlZiekt. Dienst Wageningen*, no. 58, 15.
WASEWITZ, H. (1936). Schäden durch die Blattfleckenkrankheit der Cinerarien. *Blumen- u. PflBau ver. Gartenwelt*, XL, 99–100.
WOLLENWEBER, H. W. & REINKING, O. A. (1935). *Die Fusarien.* Berlin.
ZELLER, S. M. (1927). A canker of apple and pear trees caused by *Glutinium macrosporum* n.sp. *J. agric. Res.* XXXIV, 489–96.
ZELLER, S. M. (1935). Some miscellaneous fungi of the Pacific North-west. *Mycologia*, XXVII, 463.

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AN IMPROVED TECHNIQUE FOR THE STUDY OF LIVING MYCELIUM

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Sass (1929) described the agar-film technique by which fungus mycelium was grown on microscope slides carrying a thin film of a special semi-solid, agar-containing, nutrient medium. Noble (1937) and I (1937) employed a modified technique in which the slide carrying the film was placed face downwards in a groove cut in a brass box-frame, made to measure to fit the slide, and the frame was then put in a Petri dish containing damp blotting paper. For examination under the microscope the lid of the Petri dish was removed. Light passed to the microscope lenses through the bottom of the Petri dish, the film and the slide. The lid could be replaced after examination and the fungus allowed to continue growth or, as soon as observations of interest were made on nuclear behaviour, the position on the slide was marked and noted by mechanical stage readings, the film was fixed, stained and examined and the observations made before fixation were interpreted in terms of the structures now rendered visible. The worst disadvantages of this method are the variable thickness of the Petri-dish glass which affects the light, the thickness of the slide and the cloudy nature of the medium through which the fungus hyphae have to be viewed. Only a very limited number of short lengths of hyphae can be brought into focus using a $\frac{1}{7}$ or $\frac{1}{12}$ in. oil-immersion lens, even when 'extra-thin' microscope slides are used.

The following improved technique is now being used in this Department in studying the hyphae (particularly the clamp connexions) in certain Basidiomycetes. The microscope slide to carry the film is replaced by a large glass coverslip 2 × 4 in. of the type used to cover fossil preparations, or better still by a 1 × 3 in. no. 3 or no. 2 cover-slip. The bottom of the box-frame is set on an 'extra-thin' microscope slide. The medium employed for the film is 1·5 % malt in 1·5 % British agar. As before, the films are grown in Petri-dish damp chambers. They are removed for examination resting on the brass box. The microscope slide completes the base of this small damp chamber. Films of this type have been kept growing under a $\frac{1}{16}$ in. oil-immersion lens for hours on end—sometimes overnight—before fixation or return to their Petri dishes. The small chamber is comparatively easy to manipulate and hyphae may be traced backwards from their tips up to six fields of view under either a $\frac{1}{12}$ or $\frac{1}{16}$ in. lens. By this means the formation of clamp connexions has been watched repeatedly in fungi such as *Coniophora puteana* and *Marasmius androsaceus*. In the latter species the time from the inception of the bulge on the parent hypha to the beginning of the outgrowth of a branch from the completed, fused clamp connexion is 50–60 minutes. The formation of the clamp itself takes about 20 minutes, with the greatest regularity.

Marasmius androsaceus forms its clamp connexions in full daylight—during ordinary working hours!—and the technique is simple enough for advanced students to be trusted to use the equipment and watch the process for themselves.

An account of results observed is in preparation for publication.

REFERENCES

Macdonald, J. A. (1937). A study of *Polyporus betulinus* (Bull.) Fries. *Ann. appl. Biol.* xxiv (2), 289–310.

Noble, M. (1937). The morphology and cytology of *Typhula Trifolii* Rostr. *Ann. Bot., Lond.*, N.S. 1, 67–98.

Sass, J. E. (1929). The cytological basis for homothallism and heterothallism in the Agaricaceae. *Amer. J. Bot.* xvi, 663–701.

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STUDIES ON BRITISH CHYTRIDS

II. SOME NEW MONOCENTRIC CHYTRIDS

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(With Plates IX and X and 8 Text-figures)

Few workers have been attracted to a detailed study of the aquatic Chytridiales in Great Britain (Cook, 1932; Sparrow, 1936; Ingold, 1940, 1941 and 1944), and thus the number of records for this country remains relatively small. Intensive work carried out by me on these organisms during the past two years shows that they are to be found in almost any aquatic habitat provided that a suitable substratum for growth is present. Since the Chytridiales are as yet a relatively unexplored group, the discovery of new species is not uncommon, and several are described in this paper.

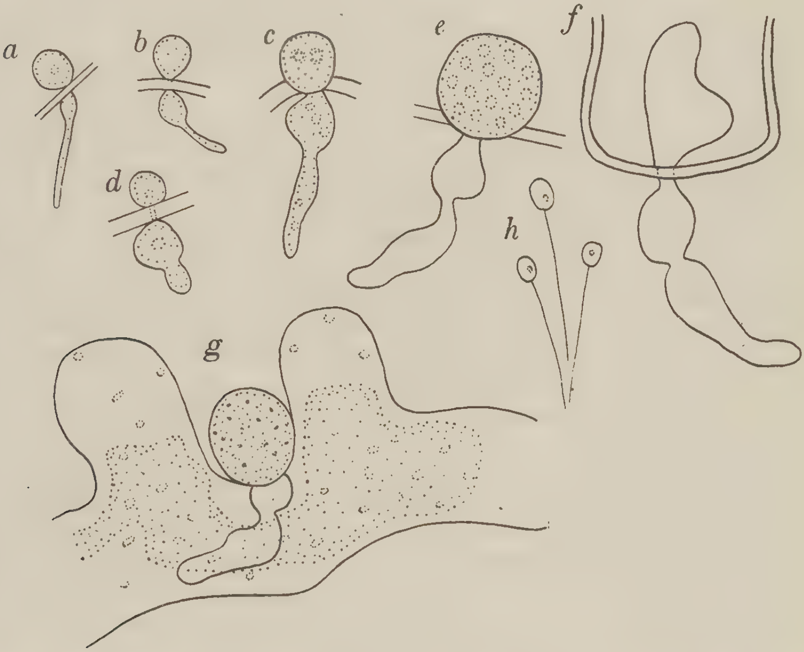
I. **Phlyctidium apophysatum** n.sp.

This chytrid was found on *Mougeotia* from the Clay Pond, Wray Castle, Windermere, in August 1945. Outgrowths from the cells are of common occurrence in *Mougeotia*, and sporangia of the fungus were often located between two such processes. The fungus is parasitic and brings about a dissociation of the host chloroplast into numerous granules.

The thallus is monocentric, and consists of an extramatrical sporangium, an intramatrical apophysis and unbranched tubular rhizoid. Young stages (Text-fig. 1 *a–d*) indicate that the apophysis is formed early, as a swelling of the germ-tube, the distal part of which develops into the tubular rhizoid. The apophysis is spherical to subspherical, and never exceeds the diameter of the sporangium. The sporangium is spherical to oval, 9–17 μ in diameter and usually produces a hundred or more zoospores, which are liberated upon deliquescence of the apex of the sporangium. The zoospores (Text-fig. 1 *h*) are oval, 1·4 × 2·4 μ, with a minute refractive globule, and a single posterior flagellum about 13 μ long; their movement is somewhat jerky. The apophysis and sporangium tend to collapse after dehiscence. Several smaller sporangia, 7–12 μ in diameter, were observed (Text-fig. 1 *e*). They contained a few regularly arranged, relatively large oil globules, but whether they actually liberate zoospores with a conspicuous globule is unknown, as dehiscence was never seen. Resting spores were not observed.

This fungus shows very clearly the dubious nature of the characteristics which have been used to separate the genera *Phlyctidium* and *Phlyctochytrium*. It resembles a species of *Phlyctidium* in the nature of its unbranched rhizoid, and a species of *Phlyctochytrium* by the possession of an apophysis.

Sparrow (1933, p. 518, Pl. 49, fig. 12) described an incompletely known fungus on *Cladophora*, probably a *Phlyctidium*, with a rhizoidal system very similar to that of the present species; *P. spinulosum* (Sparrow, 1933, p. 516, Text-figs. 1, 2) also has a slightly inflated tubular rhizoid, and it is decided to include the fungus here described in the genus *Phlyctidium* as a new species, *P. apophysatum*, taking its name from the constant, and well-developed intramatrical apophysis.



Text-fig. 1. *Phlyctidium apophysatum* n.sp. *a–d*, young thalli. *e*, sporangium possibly containing zoospores with a large oil globule. *f*, dehisced sporangium. *g*, large sporangium situated between two processes of the *Mougeotia*. *h*, zoospores. *a–f*, *h*, × 1400; *g*, × 660.

Phlyctidium apophysatum n.sp.

Thallus monocentric, consisting of an extramatrical sporangium 9–17 μ in diameter, an intramatrical apophysis, either spherical 5 μ in diameter, or subspherical $6{\cdot}7 \times 10\,\mu$, never exceeding diameter of the sporangium, continuous with a tubular rhizoid ($24 \times 7\,\mu$) to ($12 \times 3{\cdot}3\,\mu$). Zoospores oval $1{\cdot}4 \times 2{\cdot}4\,\mu$ with a minute colourless globule, and posterior flagellum 13 μ long, discharged on deliquescence of the apex of the sporangium. Resting spores not observed.

Parasitic on *Mougeotia* sp. Clay Pond, Wray Castle, Windermere, England, August 1945.

Phlyctidium apophysatum sp.nov.

Thallus monocentricus, sporangiis extramatricalibus, apophysibus et rhizoideis intramatricalibus. Sporangia 9–17 μ diam. Apophysis sphaerica 5 μ diam. aut subsphaerica 6·7–10 μ, nunquam sporangio latior.

Rhizoideum tubulare 12 × 3·3 μ ad 24 × 7 μ. Zoosporae ovales, 1·4–2·4 μ, globulo hyalino minuto, postice uniflagellatae, flagello 13 μ longo, ex apice sporangii dissoluto emergentes. Sporae perdurantes non visae.

Hab. in *Mougeotia* sp. parasiticum, Clay Pond, Wray Castle, Windermere, Anglia, August 1945.

II. A SPECIES OF *RHIZOPHIDIUM*

Scherffel (1926) described a chytrid parasitic on the sporelings of *Oedogonium* which he referred tentatively to *Rhizophidium globosum.* In February 1946 a very similar organism was found parasitizing the same host, in Clissold Park Lake, London.

From one to fourteen individuals of the parasite may occur on a single sporeling, and when only one is present a characteristic curvature is induced in the host cell, the chloroplast of which is converted into a mass of brown granules (Text-fig. 2*c* and Pl. IX, fig. 4).

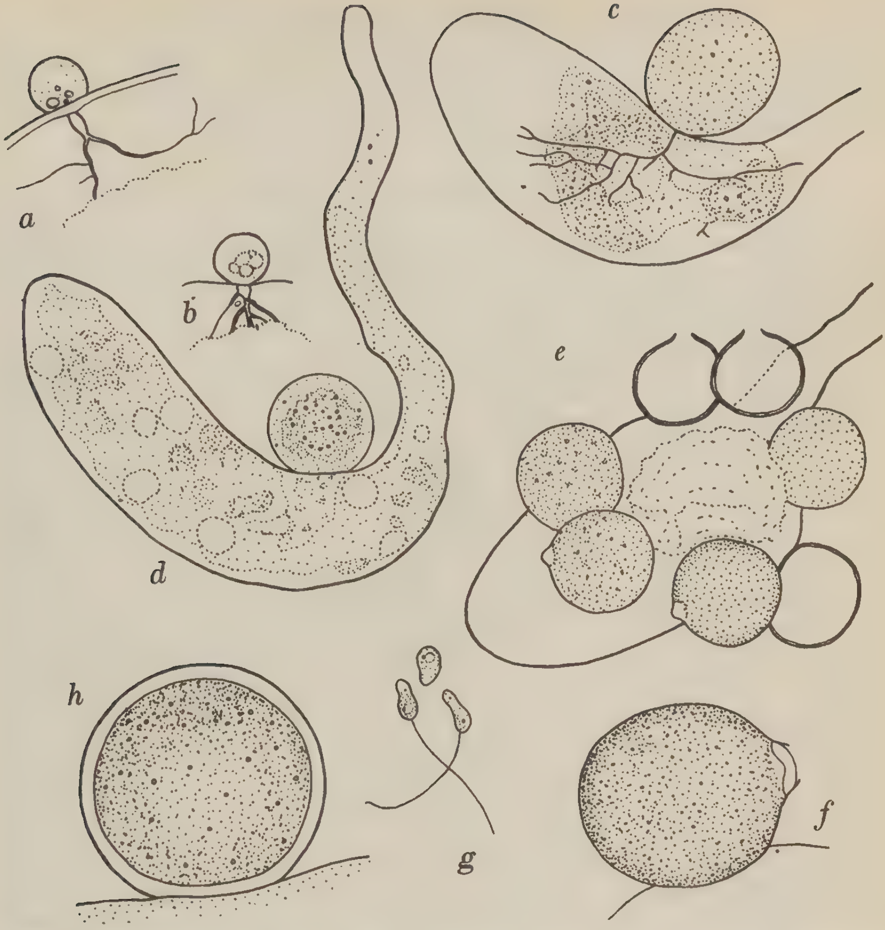
The rhizoidal system is often difficult to observe owing to the dense chloroplast of the host, but where visible it consists of a main axis, rarely slightly swollen immediately beneath the host wall (Text-fig. 2*a*, *b*), which branches to give a meagre rhizoidal system. The spherical sporangia vary in size from 7·5 to 34·3 μ in diameter, and where many occur on a single host cell they are relatively small.

As the sporangium matures a single lateral dehiscence papilla becomes visible, and it appears to be filled with a plug of highly refractive material. The part of the sporangium wall forming the papilla deliquesces, but whether the plug also deliquesces or is extruded as a solid mass is unknown, as the actual moment of dehiscence was never observed. Hundreds of posteriorly uniflagellate zoospores 4 μ long, 2 μ diameter, are produced. They are of unusual structure for a chytrid since they have no conspicuous oil globule, but they contain one or two minute, highly refractive granules, often situated laterally near the posterior end (Text-fig. 2*g*).

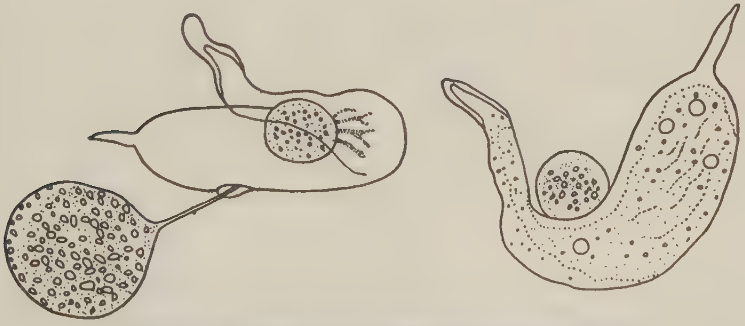
Spherical, asexually formed resting spores 21·4–35 μ in diameter were seen, which appeared to produce little effect on the host chloroplast (Text-fig. 2*h* and Pl. IX, fig. 6). They are similar to the zoosporangia, but the wall is up to 2 μ thick; their contents are at first granular, but later become oily. Neither the rhizoids nor germination of these resting spores was seen; one empty specimen showed a single lateral dehiscence pore.

The chytrid here described agrees in all essentials, except one with Scherffel's organism. In the latter the rhizoidal system is stated to consist of a few fairly thick but not long, forked rhizoids, arising from the base of the sporangium (Text-fig. 3), whereas in the London material there is a single main axis. In my opinion this is not a significant enough difference to warrant a separation of the two forms.

Although the chytrid here described is very similar to *R. globosum,* according to Sparrow's (1943) definition it cannot find its true place in this species owing to the smooth-walled resting spore. Great difficulties are presented in dealing with the globose species of *Rhizophidium.* The records are numerous, but only rarely is a complete description given. Observations on the structure and method of formation of the resting spores is of



Text-fig. 2. *Rhizophidium* sp. *a*, *b*, very young sporangia; intramatrical rhizoid is slightly swollen immediately within the host wall. *c*, sporangium with well-developed rhizoidal system. *d*, immature sporangium rhizoids not visible; characteristic curvature of the host present, but contents little disorganized. *e*, mature and dehisced sporangia. *f*, mature sporangium with lateral plug of highly refractive material. *g*, zoospores. *h*, smooth walled resting spore. *b*, *e*, *h*, *f*, ×975; *a*, *g*, ×1333; *c*, ×700; *d*, ×700.



Text-fig. 3. *Rhizophidium globosum* (after Scherffel, 1926).

utmost importance, and when investigations have been carried out on the specificity and morphological variations of these organisms, a thorough revision of the genus will be necessary.

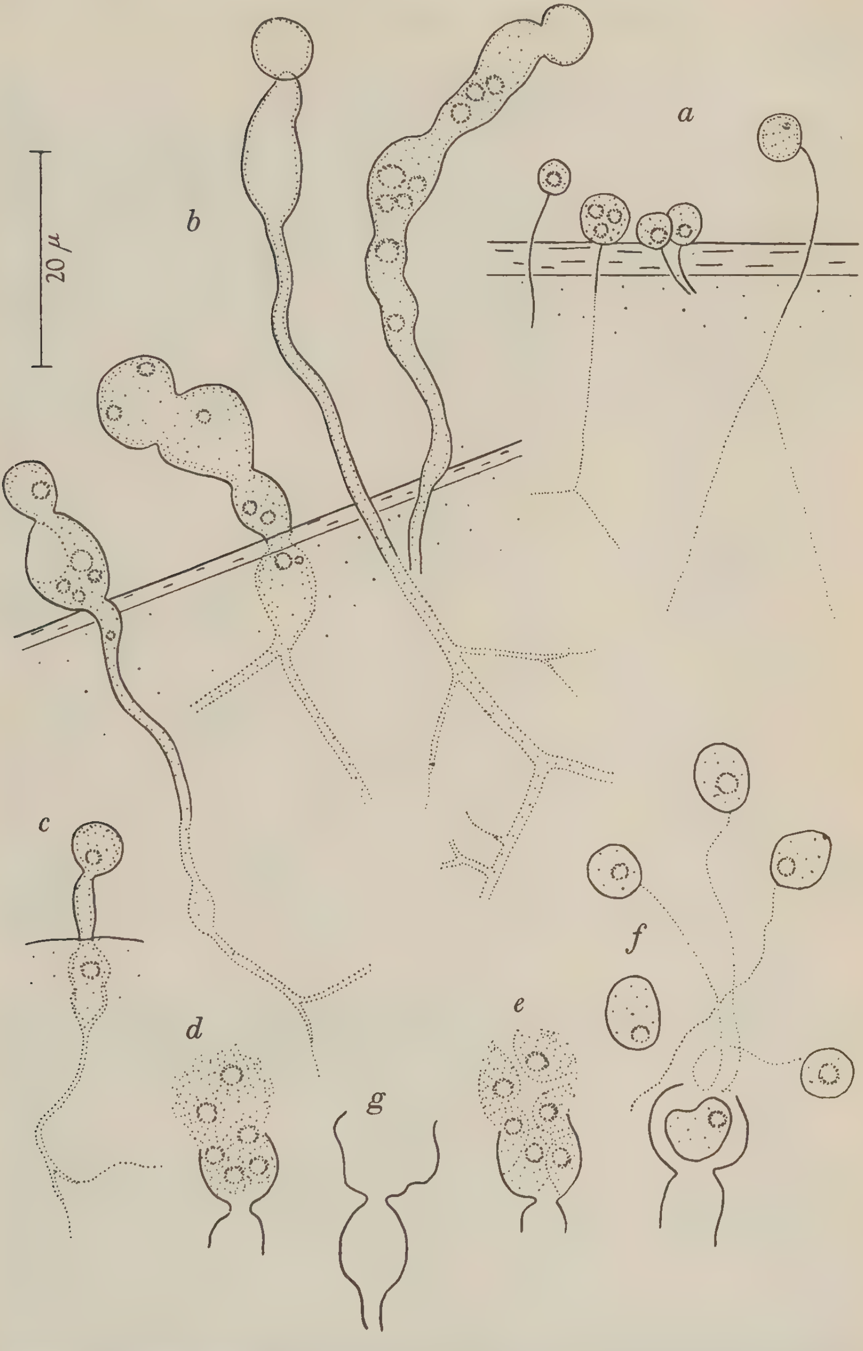
III. **Rhizidium variabile** n.sp.

This fungus was found growing as a saprophyte on *Spirogyra* in May 1945 and 1946, from a pond in Chelsea Physic Garden, London. It appeared after the *Spirogyra* had been kept in the laboratory for some weeks, by which time the algal cells were almost unrecognizable.

The thallus is monocentric, consisting of an extramatrical, usually unbranched system of diverse form and size, and an intramatrical ultimately branched rhizoidal system. The extramatrical part is composed of the body of the encysted zoospore, more or less swollen, continuous with a single stout rhizoid, which may exhibit one or several swellings throughout its length. The zoospore on germination produces a germ tube which branches distally, the swellings developing later. The diversity of the thallus structure is illustrated in Text-fig. 4*b* and Pl. X, figs. 4–6. The exact point where the thallus enters the host cell is often difficult to determine, because of the disorganization of the cell walls. In the material found in 1945 (Text-fig. 4*c*) the intramatrical system of the chytrid was rarely swollen, whereas in the collection made a year later many such specimens were seen (Text-fig. 5*c*, *e*) which simulated species of the genus *Phlyctochytrium*. During the development of the vegetative part of the thallus the encysted zoospore remains small, later there accumulates in it the protoplasmic contents of the whole thallus, and it enlarges to form a spherical or subspherical sporangium, 6–13 μ in diameter, which contains from one to twelve relatively large zoospores. These exude in a motionless mass on deliquescence of the apex of the sporangium. At first they are amoeboid, but soon round off and swim away. The zoospores are 4·4–5 μ in diameter with an oil globule 1–2 μ in diameter and a single posterior flagellum 26 μ long. The sporangium wall collapses after dehiscence. Resting spores were not observed.

In general structure this species most nearly resembles *Rhizidium mycophilum* Braun, previously recorded from England by Sparrow (1936), growing on exuviae of Chironomidae. However, the series of subsporangial swellings is not a feature of *R. mycophilum*, and the sporangia and number of zoospores produced are much smaller. Further, the zoospore mass does not exhibit the changes in shape that were recorded for Sparrow's material of *R. mycophilum*. Karling (1944) recognizes *R. mycophilum* Braun as two species, *R. mycophilum* Braun and *R. Nowakowskii* Karling (= *R. mycophilum* Nowak), based on differences in habitat, in the structure of the zoospores, and in the resting sporangia. As neither Sparrow (1936) nor I have observed resting spores the exact affinity of the present species cannot be determined.

It is suggested that the present fungus shall be described as a new species, until experiments have been carried out on the morphological variations which occur in single-spore cultures on different hosts in *R. variabile* or its



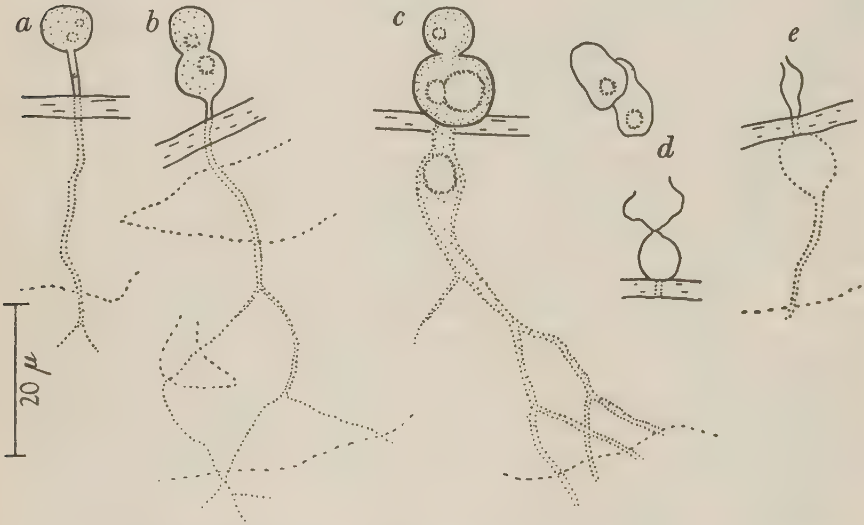
Text-fig. 4. *Rhizidium variabile* n.sp. *a*, germinating zoospores on *Spirogyra* sp. *b*, variations in thallus structure. *c*, thallus with endobiotic swelling. *d–g*, stages in dehiscence of sporangia. (All from Chelsea Physic Garden, 1945.)

near allies, after which it may be necessary to revise the position of this species.

Rhizidium variabile n.sp.

Thallus monocentric, extramatrical part consisting of a sporangium 6–13 μ in diameter (developed from the encysted zoospore), and a single stout rhizoid, which may have one or more swellings. Intramatrical part sometimes swollen, tapering to a branched rhizoidal system. Sporangium containing 1–20 zoospores which emerge, surrounded by a vesicle, on deliquescence of the sporangial apex. Zoospores spherical 4·4–5 μ in diameter, with a single oil globule 1–2 μ in diameter, and a posterior flagellum 26 μ long. Sporangium wall collapsing after dehiscence. Resting spores not observed.

On dead *Spirogyra* sp., Chelsea Physic Garden, London, England.



Text-fig. 5. *Rhizidium variabile* n.sp. *a*, *b*, young thalli with slender branched intramatrical rhizoidal system. *c*, thallus with extramatrical and intramatrical apophyses. *d*, empty sporangium, above two zoospores. *e*, empty collapsed sporangium. (Chelsea Physic Garden, 1946.)

Rhizidium variabile sp.nov.

Thallus monocentricus. Pars extramatricalis e sporangio et rhizoideo nico dilatationibus singulis vel pluribus praedito consistens. Pars intra-natricalis interdum inflata, in rhizomycelium ramosum attenuata. porangium 6–13 μ diametro, zoosporas 1–20 includens. Zoosporae haericae, 4·4–5 μ diam., globulo unico hyalino 1–2 μ, postice uniflagel-tae, flagello 26 μ longo, vesiculo inclusae emergentes. Membrana orangii post dehiscentiam collabit. Spore perdurantes non visae.

Hab. in *Spirogyra* sp. emortua, Chelsea Physic Garden, London, Anglia.

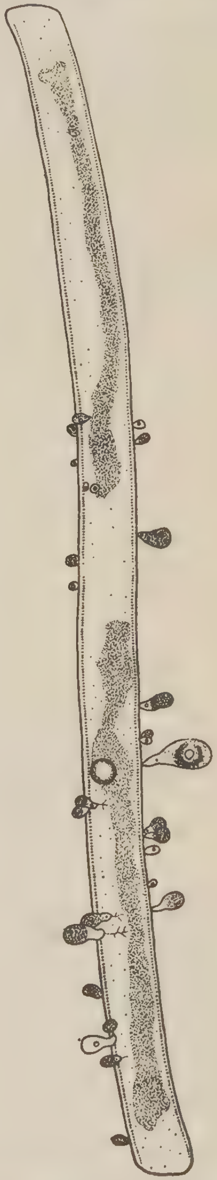
IV. CHYTRIDIUM VERSATILE VAR. ACAULIS

This chytrid (Text-fig. 7, and Pl. X, fig. 7, 8) was found growing on *Nitzschia sigmoidea* (Ehrenb.) W.Sm., in Bradbourne Park Lake, Sevenoaks, Kent, England, from November 1944 to March 1945. It was also found in a collection from the River Yeo, Sherborne, Dorset, in March 1945.

Although other diatoms were abundant from both localities (e.g. species of *Pinnularia*, *Synedra*, *Navicula*, *Surirella*, *Melosira* and *Gyrosigma*), they were never attacked. It therefore seems probable that the chytrid is specific to *Nitzschia sigmoidea*, as there should have been ample opportunity for infection to occur in the small flocculent masses into which these diatoms were crowded. The fungus is parasitic, but the contents of the infested cells are little affected; the chromatophore retracts somewhat at the point of entry of the rhizoids, but the movement of the host is not impaired. An infected diatom may carry from one to thirty individuals of the parasite (Text-fig. 6).

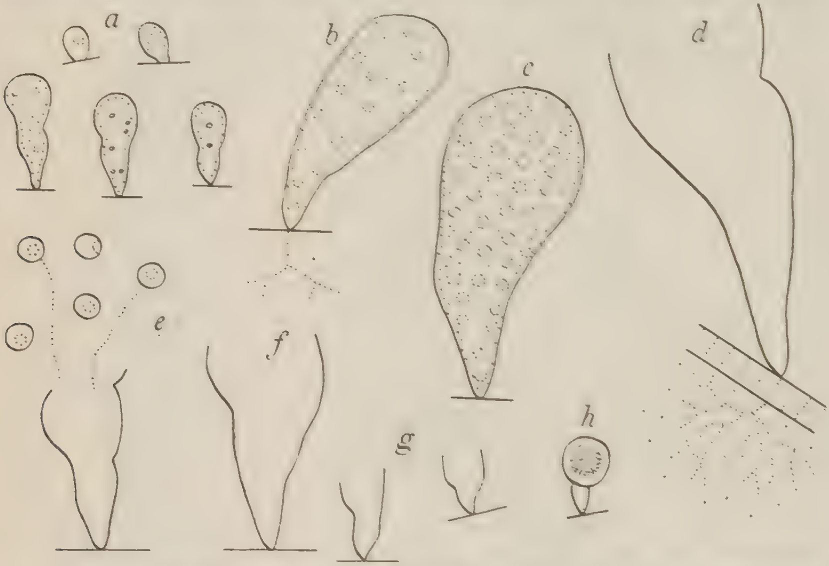
The zoospore encysts on the surface of the diatom and produces a germ tube which usually penetrates a carinal dot. From the base of the germ tube a branched intramatrical rhizoidal system arises (Text-fig. 7*d*). The mature sporangia are obpyriform and taper to a knob-like base sessile on the host. The sporangium wall is smooth, colourless, thin distally, often becoming thickened at the base. The sporangia range from 4 to 25 μ in diameter, and from 15 to 60 μ high; while the smaller sporangia contain a few zoospores, the larger ones may produce eighty or more. The zoospores are spherical, 3–4 μ in diameter, uniguttulate with a single posterior flagellum, and upon the detachment of an apical, rarely somewhat lateral operculum, they escape in a mass from the sporangium but quickly separate, and glide away individually. One probable resting spore was observed (Text-fig. 7*h*), but it is thought unwise to base a description on this specimen alone.

Apart from the absence of a stalk, its larger size and supposed specificity for *Nitzschia sigmoidea*, the fungus agrees well with *Chytridium versatile* Scherffel, already recorded from this



Text-fig. 6. *Chytridium versatile* var. *acaulis*. *Nitzschia sigmoidea* bearing numerous chytrid thalli in various stages of development. × 330.

country by Sparrow (1936). As in *C. versatile* (see Scherffel, 1926) the sporangium bends back as the diatom pushes against debris in its environment, in spite of the fact that this species is sessile. After the obstruction is passed the sporangium snaps back to its original upright position. Scherffel (1926, Plate 9, figs. 19, 20) figures three sporangia apparently without a stalk; however, no mention is made in the text to such sessile forms. Owing to the absence of any major structural differences from *C. versatile* Scherffel it is proposed to erect a new variety, **C. versatile** var. **acaulis**, being characterized by the absence of a stalk.



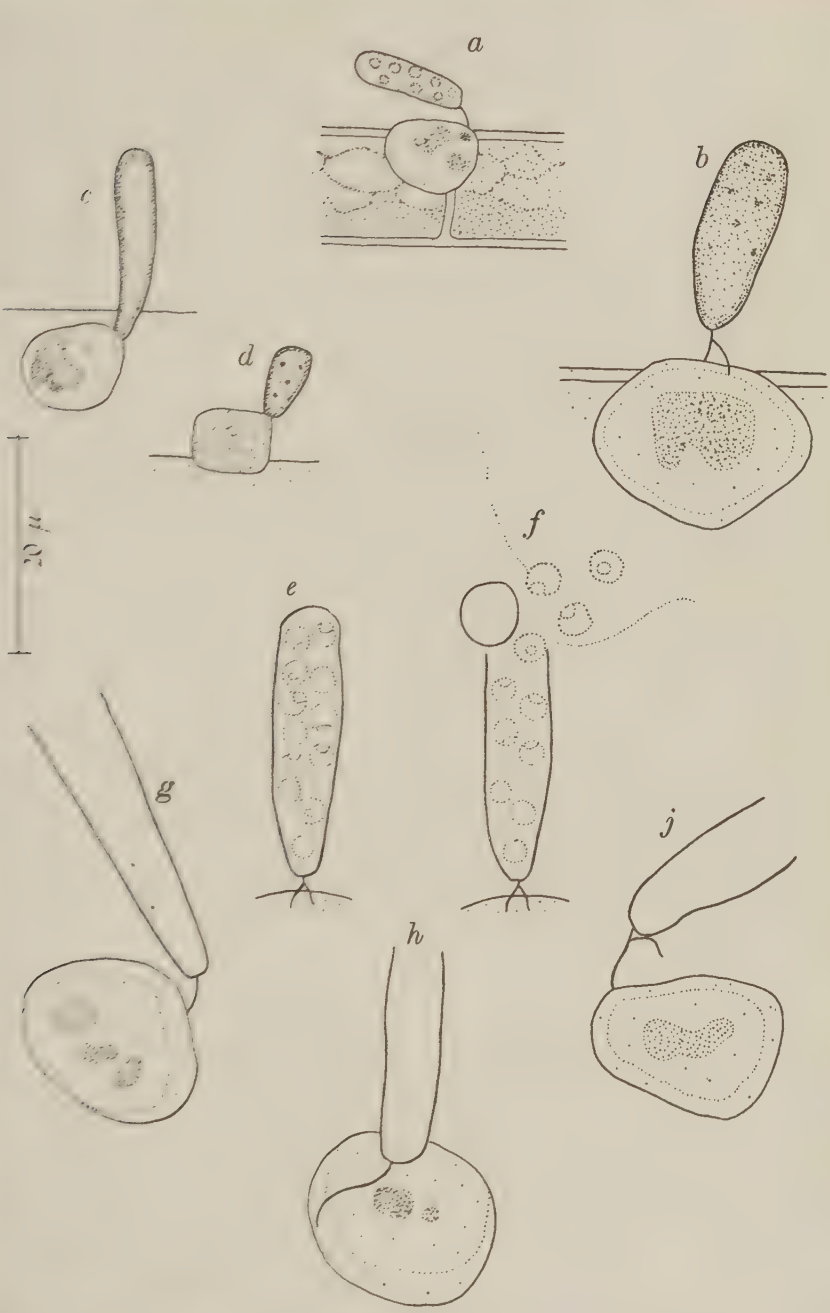
Text-fig. 7. *Chytridium versatile* var. *acaulis*. *a*, young sporangia. *b*, sporangium inclined to long axis of host cell. *c*, mature sporangium. *d*, dehisced sporangium showing branched rhizoidal system. *e*, zoospores. *f*, *g*, dehisced large and small sporangia. *h*, resting spore (?). *c*, *d*, × 1400; *a*, *b*, *e*, *f*, *g*, *h*, × 660.

V. **Chytridium cocconeidis** n.sp.

This fungus was found on *Cocconeis pediculus* Ehrenb., itself epiphytic on *Cladophora*, in a small lake in Clissold Park, London, March 1945. Sparrow (1943) records no chytrid on *Cocconeis*, and the organism appears to be an undescribed species.

Judging by the disorganized state of the chromatophores in the affected diatoms, the chytrid is parasitic. It seems to be limited in its host range, since *Rhoicosphenia curvata* (Kg.) Grun., which was also abundant on the *Cladophora*, was not attacked.

The species is monocentric, and the thallus consists of a cylindrical sporangium, and a rhizoidal system. This system consists of an extramatrical part, and presumably also of an intramatrical part, although this was not demonstrated microscopically. The extramatrical part consists sometimes of a straight unbranched portion (Text-fig. 8*a*, *g*, *h*), but at



Text-fig. 8. *Chytridium cocconeidis* n.sp. *a*, *b*, sporangia with an extramatrical rhizoidal system. *c*, *d*, mature apparently sessile individuals. *e*, *f*, mature and dehisced sporangium. *g*, *h*, *j*, empty sporangia.

other times it is forked near the attachment to the host cell (Text-fig. 8*b*, *e*). Occasionally no extramatrical part was visible, and the sporangium appeared to be sessile on the *Cocconeis* (Text-fig. 8*c*, *d*). Mature sporangia measure 15–29 μ in length, by 5–6 μ in diameter, and are sometimes inclined at an angle to the main extramatrical rhizoid. The sporangium contains from twelve to thirty zoospores and dehisces by the separation of a convex apical lid 2–3 μ in diameter. Upon detachment of the lid several zoospores escape together, but the remainder emerge singly. The zoospores are spherical, 2–3 μ in diameter, with a conspicuous refractive globule and a single posterior flagellum. Their movement is predominantly hopping, with periods of gliding. The method of infection of the *Cocconeis* by the zoospore was not satisfactorily demonstrated. Resting spores were not observed.

Since this chytrid is operculate, in Sparrow's classification (1943) it belongs to the Chytridiaceae. The only possible genus appears to be *Chytridium*, but this genus contains no species with a branched extramatrical rhizoidal system, although a few species, *C. versatile* Scherffel, *C. curvatum* Sparrow, and *C. Lagenula* Braun pro parte, may have a short slender extramatrical stalk.

Although the inclusion of this species in the genus *Chytridium* may necessitate a slight extension of the concept of the genus, this is nevertheless preferable to the erection of a new genus. The species *C. cocconeidis* is proposed, taking its name from the host upon which it is apparently a specialized parasite.

Chytridium cocconeidis n.sp.

Thallus monocentric, eucarpic, sporangium extramatrical cylindrical 15–29 μ in length, 5–6 μ in diameter, dehiscing by an apical lid, and containing 12–30 zoospores. Zoospores spherical 2–3 μ in diameter, with a colourless globule and single posterior flagellum. Extramatrical rhizoidal system simple or branched 2–9 μ in length, rarely absent. Intramatrical rhizoidal system not observed. Resting spores not observed.

On living cells of *Cocconeis pediculus* from Clissold Park, London, England, March 1945.

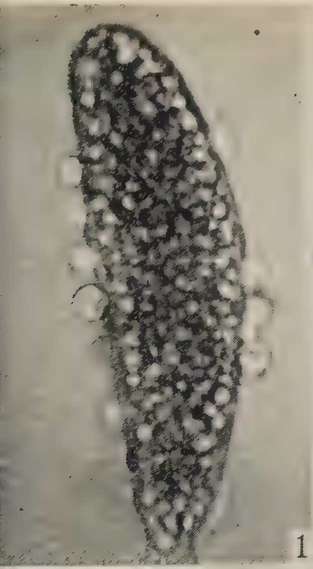
Chytridium cocconeidis sp.nov.

Thallus monocentricus, eucarpicus. Sporangia extramatricalia, cylindrica, 15–29 × 5–6 μ, operculo apicale dehiscentia, 12–30 zoosporas includentia. Zoosporae sphaericae 2–3 μ diam., globulo hyalino refractivo, postice uniflagellatae. Rhizoidea extramatricalia, simplicia vel ramosa, 2–9 μ longa, raro nulla. Sporae perdurantes non visae.

Hab. in cellulis vivis *Cocconeidis pediculi*, Clissold Park, London, Anglia, Martio 1945.

SUMMARY

Five monocentric chytrids are described growing on algae from Great Britain. Of these, three are new species, namely, *Phlyctidium apophysatum*, *Rhizidium variabile* and *Chytridium cocconeidis*. *Chytridium versatile* var. *acaulis*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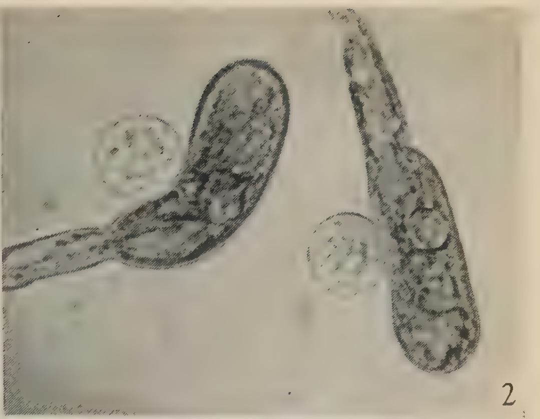
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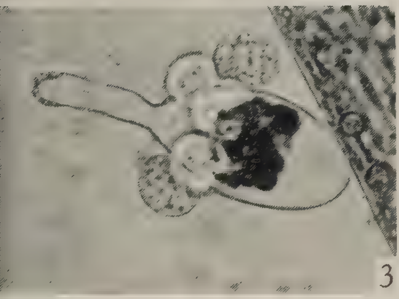
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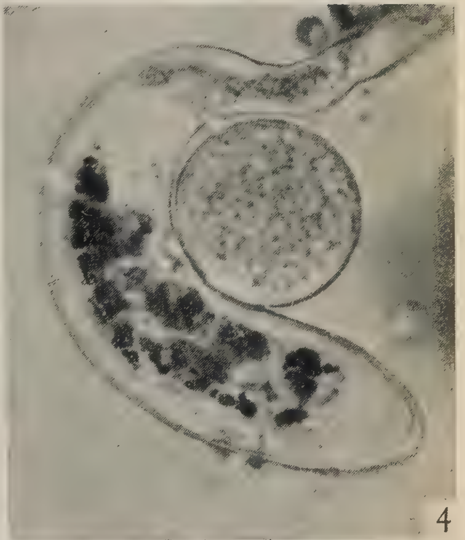
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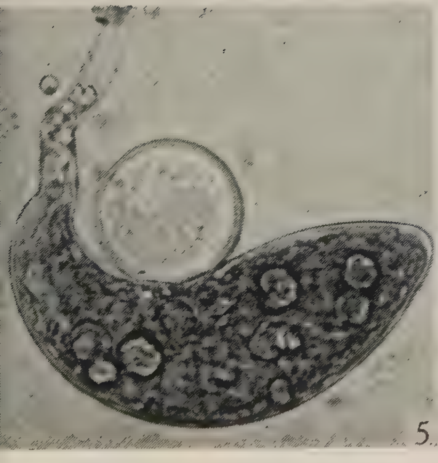
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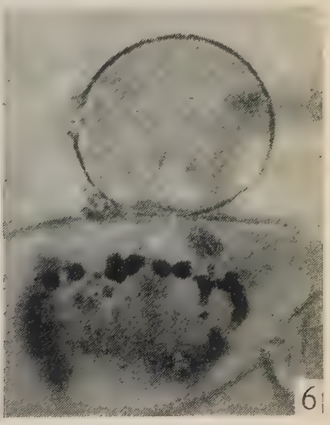
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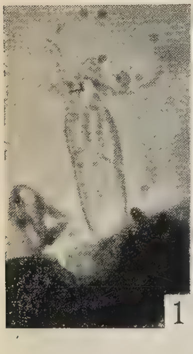
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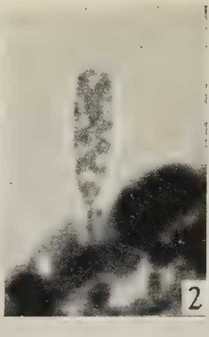
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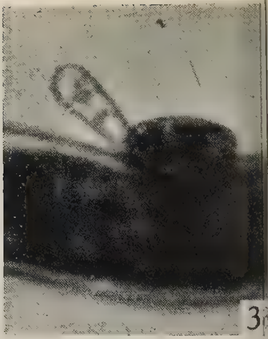
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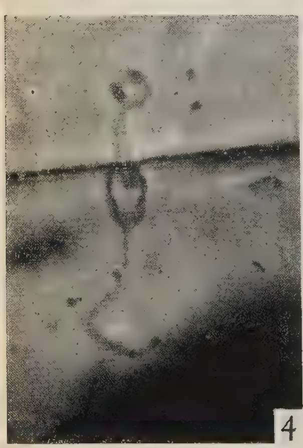
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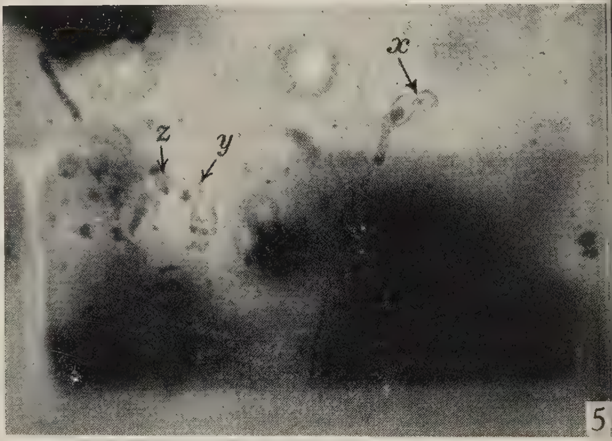

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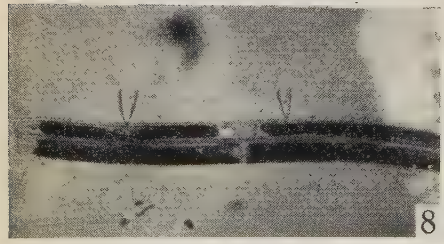
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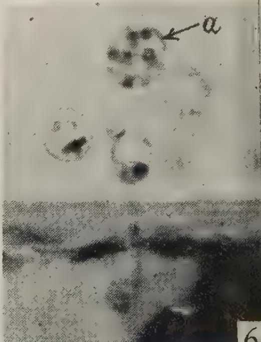

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represents a new variety, and a species of *Rhizophidium* is identified with *Rhizophidium globosum* Scherffel (1926).

My thanks are due to Miss E. M. Wakefield for the Latin diagnoses and to Prof. C. T. Ingold for the advice he has given throughout the course of this work.

REFERENCES

Cook, W. R. I. (1932). An account of some uncommon British species of the Chytridiales found in algae. *New Phytol.* xxxi, 133–44.

Ingold, C. T. (1940). *Endocoenobium Eudorinae*, gen. et sp.nov., a chytridiaceous fungus parasitizing *Eudorina elegans* Ehrenb. *New Phytol.* xxxix, 97–103.

Ingold, C. T. (1941). Studies on British Chytrids. I. *Phlyctochytrium proliferum* sp.nov. and *Rhizophidium Lecythii* sp.nov. *Trans. Brit. mycol. Soc.* xxv, 41–8.

Ingold, C. T. (1944). Studies on British Chytrids. II. A new Chytrid on *Ceratium* and *Peridinium*. *Trans. Brit. mycol. Soc.* xxvii, 93–6.

Karling, J. S. (1944). Brazilian chytrids II. New species of *Rhizidium*. *A. J. Bot.* xxxi, 254–60.

Scherffel, A. (1926). Einiges über neue oder ungenügend bekannte Chytridineen (Der 'Beiträge zur Kenntnis der Chytridineen', Teil II). *Arch. Protistenk.* liv, 167–260.

Sparrow, F. K. (1933). Inoperculate chytridiaceous organisms collected in the vicinity of Ithaca, N.Y., with notes on other aquatic fungi. *Mycologia*, xxv, 513–35.

Sparrow, F. K. (1936). A contribution to our knowledge of the aquatic Phycomycetes of Great Britain. *J. Linn. Soc. Lond.* (*Bot.*), l, 417–78.

Sparrow, F. K. (1943). *Aquatic Phycomycetes.* Ann Arbor, U.S.A.: University of Michigan Press.

EXPLANATION OF PLATES

Plate IX

Rhizophidium sp.

Fig. 1. Many germinated zoospores on an *Oedogonium* sporeling. × 700.

Fig. 2. Two sporelings each with a young sporangium; the one on the right already shows a slight curvature. × 650.

Fig. 3. Sporeling attacked by five sporangia, the chloroplast is very much contracted.

Fig. 4. Almost mature sporangium. × 650.

Fig. 5. Sporangium showing lateral region of dehiscence, the outer wall has already deliquesced. × 650.

Fig. 6. Resting spore with thick wall; the host contents are not disorganized. × 650.

Plate X

Fig. 1. *Chytridium cocconeidis*; dehisced sporangium with zoospores at its apex. × 480.

Fig. 2. Sporangium with needle-like extramatrical rhizoid. × 640.

Fig. 3. Cylindrical, apparently sessile sporangium on *Cocconeis pediculus*. × 960.

Fig. 4. *Rhizidium variabile*; young thallus showing encysted zoospore, intramatrical apophysis and rhizoidal system. × 560.

Fig. 5. Thalli visible at (x, y, z). (x) shows a long extra matrical rhizoid; the cell walls of the *Spirogyra* are just out of focus. × 280.

Fig. 6. At (a) is a mature sporangium with the oil globules of six zoospores clearly delimited; a young thallus is visible to the right of (a). × 736.

Fig. 7. *Chytridium versatile* var. *acaulis*, a mature sporangium on *Nitzschia sigmoidea*. × 700.

Fig. 8. Two dehisced sporangia. × 190.

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THE USE OF PERFORATED CARDS FOR PRELIMINARY IDENTIFICATION OF FUNGI

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(With 2 Text-figures)

Introduction

The use of perforated cards for the rapid identification or selection of individuals possessing any particular combination of characteristics is now well known. The method has been successfully used for the identification of timbers from their microscopic structure and other features (Clarke, 1938; Phillips, 1941). An attempt has now been made to construct a perforated card key for the rapid preliminary identification of Hymenomycetes belonging to Polyporaceae and Agaricaceae.

Many would-be students of these groups are discouraged by the initial difficulties which they experience when they attempt to identify specimens by means of the orthodox methods, such as dichotomous keys based on a few characters which may appear to them ill-defined. Nothing encourages students more than to make by themselves a number of successful determinations, and it is with the hope of encouraging them to take an interest in the higher Basidiomycetes that the following suggestions are published.

Method

Cards with small marginal perforations are marked out with a list of the most useful diagnostic features. Cards printed with a list of these features may be prepared* or the features may be listed only on a master card and corresponding blank cards prepared for each species to be included in the key. The cards are prepared for use by clipping out the edge of the holes opposite to the number assigned to these particular features that are possessed by the species in question; for instance, if a species is white, soft and possesses a fixed ring on the stem the holes assigned to these characters are clipped out. All the cards of the group that is under consideration are stacked together in a box, *the right way up and facing the same way*, one clipped corner of the card providing a check that this is done. It is then immaterial how they are arranged in the 'pack'.

When using the key the unknown specimen is examined and the most important diagnostic feature is noted. A knitting needle, preferably mounted in a wooden handle, is then run through the hole through the entire pack of cards, which is gently but thoroughly shaken. All the cards describing species which possess this feature fall out; a second well-marked diagnostic feature is then chosen and the needle passed through the corresponding hole in the cards which have fallen out, the process being continued until one card or only a few cards are left. If only a few cards are left it is then usually possible to select the card descriptive of the unknown specimen, since additional notes on characteristic features that are not included in the standard characters assigned to the perforations are written on the cards to assist final diagnosis.

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* The right to produce cards of this kind is covered by patent held by Messrs Copeland Chatterson and Co., London, E.C. 4.

The successful working of any key depends, of course, on the choice of well-defined, constant characters on which to base it.

It is recognized that one of the principal difficulties in satisfactorily describing fungi for purposes of subsequent recognition is the paucity of well-marked characters—shape, size and colour often being so variable as to afford little assistance, whilst other more constant characters, such as texture, are difficult to describe precisely. Such difficulties arise in the construction of any type of diagnostic key, but the perforated card system provides a loophole by which variations can be covered, e.g. if a species is black or white, according to conditions under which it is grown, both of the holes corresponding to these characters are clipped so that the card for this species falls out whether the specimen belongs to the black or the white variety, and its card will be among those chosen for further selection.

ADVANTAGES OF PERFORATED CARD SYSTEM

(1) Any striking characteristic may be chosen to make the first selection, whereas with a dichotomous key the choice of characters and the sequence in which they are made must follow the lines laid down by the author of the key. A few examples will make this clear. In practice one often recognizes a species by its odour or by its change of colour on wounding—such features cannot obviously be made the basis of a formal dichotomous key, but using the perforated cards one can at once select those species having *any* such striking characteristics.

(2) Card key systems permit the inclusion of species with features untypical of the genera to which they belong which have to be treated as exceptions in dichotomous keys: for instance, there are species that possess coloured spores that are normally included in general like *Lactarius* and *Russula* which in some classifications (e.g. Ramsbottom, 1923) are described for the sake of simplicity as possessing white spores.

(3) New species can readily be introduced into the key without affecting the position of those already included.

(4) The number of species included may amount to many hundred without rendering the key unworkable.

While the number of alternative characters that can be included in a card key cannot conveniently exceed 120–130, the blank space in the centre of the card can be used on which to write supplementary information.

The general keys to the Polyporaceae and Agaricaceae illustrated below are intended to serve as guides only to well-marked species or to groups of species. In some genera they will obviously serve only to indicate the groups of species that some taxonomists will regard as subgenera or genera. I wish to emphasize that it would not usually be possible, for instance, to run down a species of *Cortinarius* or *Russula* from a general key to the Agaricaceae of any region, but the key should lead to the group of the particular genus, which can then be studied in detail from a recognized flora. It should be possible for anyone interested in one of these difficult groups to construct for themselves a card key restricted to that particular genus in which the characteristics of the species in the genus are given in greater detail than is possible in the general key. For instance, the diagnostic characters of the species of *Boletus* as described by Pearson (1946) could well be used as the basis on which to construct a perforated card key.

Use of perforated card key

It is obviously necessary for the user of a card key to be familiar with the features listed and to have some idea of their relative importance for diagnostic purposes. In using the key for Agaricaceae one would naturally choose first such features as spore-colour, attachment of gills, texture of pileus and stem, and so on. The characters printed on the card are the main basic characters, those assigned to the holes lettered *a*, *b*, *c*, etc., are those which amplify or extend the range of the main characters. On the Agaricaceae card, *a–f*, for instance, are additional colours, *g–l* give details of gills, *m–r* of spores and hymenium, while *s–y* describe the upper surface of the pileus in greater detail.

When using the key it is important to realize that some of the features are to be understood as relative to the size of the fungus, for instance, 'flesh thin' or 'flesh thick' is not an absolute quantity in the way that the overall size is—such features should be used only after the more definite characters have been employed.

Fig. 1 shows a sample card from the card key to the Agaricaceae clipped to describe *Amanita muscaria*; for this readily recognized species three features are sufficient to sort out this particular card from the pack, (1) the volva, (2) the fixed ring, (3) the colour of the pileus.

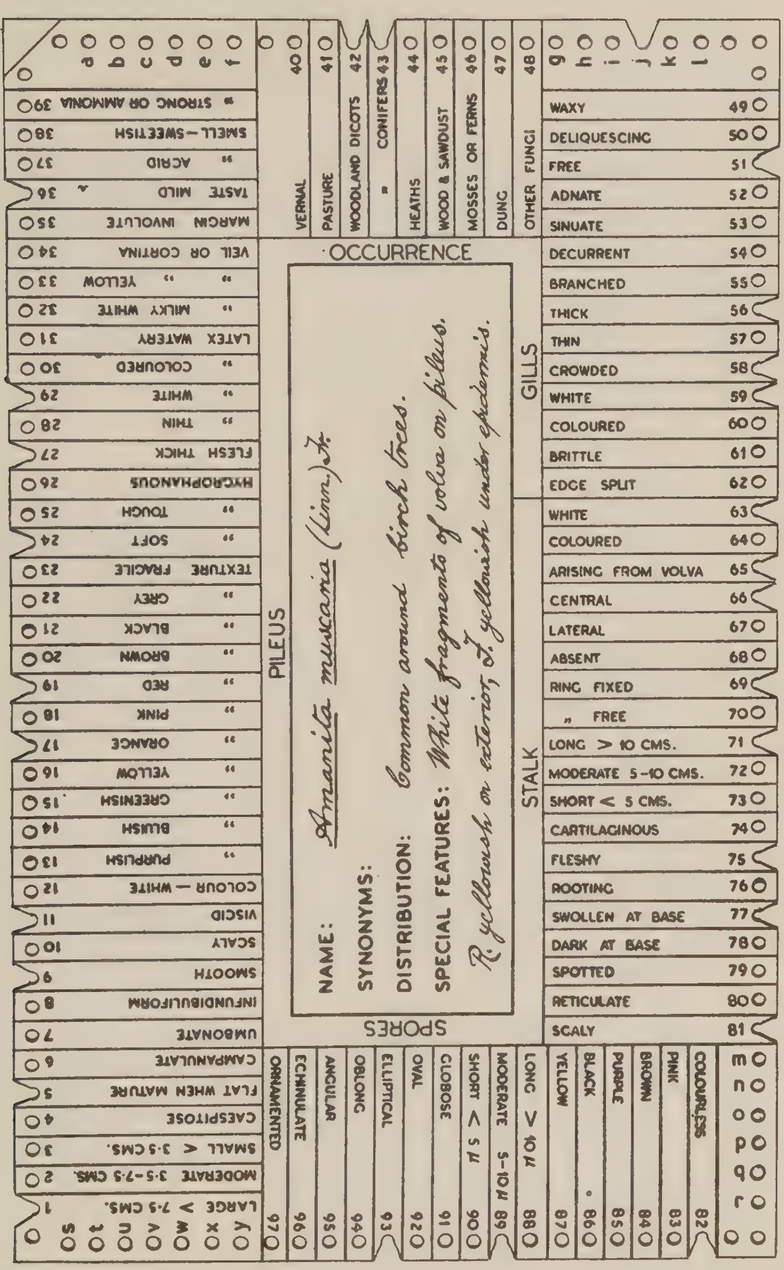
Subsidiary characters not printed on the card for Agaricaceae:

a. Pileus fuscous
b. Pileus ferruginous
c. Pileus yellow-brown
d. Pileus olivaceous
e. Pileus fawn or bistre
f. Pileus pallid or off-white

g. Gills adnexed
h. Gills emarginate
i. Gills ventricose
j. Gills having irregular edges
k. Gills having crisped edges
l. Gills anastomosing or connected by veins

m. Spores pip-shaped
n. Spores curved-allantoid
o. Spores apiculate
p. Spores guttulate
q. Spores amyloid
r. Cystidia present

s. Upper surface of pileus tomentose
t. Upper surface of pileus pruinose
u. Upper surface silky or fibrillose
v. Flesh blueing on wounding
w. Flesh reddening on wounding
x. Pileus umbilicate
y. Pileus repand



NAME: *Amanita muscaria* (Linn.) Fr.

SYNONYMS:

DISTRIBUTION: Common around birch trees.

SPECIAL FEATURES: White fragments of volva on pileus.

R. yellowish on exterior, J. yellowish under epidermis.

Fig. 1.

SPOROPHORE

No.	Character
1	Annual
2	Perennial
3	Long > 15 cm.
4	Mod. 5–15 cm.
5	Short < 5 cm.
6	Thick > 5 cm.
7	Mod. 1–5 cm.
8	Thin < 1 cm.
9	Imbricated
10	Stalk central
11	Stalk lateral
12	Stalk black at base
13	Sessile
14	Resupinate
15	Hard, woody
16	Tough
17	Soft
18	Upper surface white
19	Upper surface brown
20	Upper surface yellow-brown
21	Upper surface yellow
22	Upper surface greyish black
23	Upper surface purplish pink
24	Upper surface white, reddening
25	Upper surface white, blueing
26	Upper surface shaggy or scaly
27	Upper surface villose-velvety
28	Upper surface smooth
29	Upper surface polished
30	Upper surface zoned
31	Sclerotium attached
32	Pronounced odour

SPORES

No.	Character
33	Colourless
34	Coloured
35	Long > 10 μ
36	Mod. length 5–10 μ
37	Short < 5 μ
38	Spherical
39	Ovoid
40	Oblong, cylindrical
41	Elliptical
42	Allantoid
43	Truncate
44	Rough-walled
45	Thick-walled
46	Thin-walled

CONTEXT

No.	Character
47	Dark brown
48	Coloured, pale
49	White

HYMENIUM

No.	Character
50	Gills
51	Spines
52	Daedaleoid
53	Trametoid
54	Fistuline
55	Pores large
56	Pores small
57	Pores hexagonal
58	Pores torn irregular
59	Pores light colour
60	Pores dark colour
61	Pores glistening
62	Tubes homogeneous
63	Tubes heterogeneous
64	Tubes long — 10 mm.
65	Tubes mod. 3–10 mm.
66	Tubes short — 3 mm.
67	Tubes stratified
68	Margin sterile
69	Clamps present
70	Setae present
71	Cystidia present
72	Cystidia crowned
73	Hyphae parallel
74	Hyphae thick-walled
75	Secondary spores

OCCURRENCE

No.	Character
76	Living trees
77	Felled wood, stumps
78	Soil and roots
79	Trunk
80	Small branches
81	Sapwood
82	Heartwood
83	Coniferae
84	On Monocotyledons
85	On Dicotyledons
86	On Fagaceae
87	On Salicaceae
88	On Betulaceae
89	On Leguminosae
90	On Rosaceae
91	Oleaceae
92	On Ulmaceae

ROT

No.	Character
93	Brown-cubical
94	White, diffused
95	White, pocket
96	Zone lines, black
97	Zone lines, coloured
98	Mycelial sheets

a
b
c
d
e

NAME:
SYNONYMS:
DISTRIBUTION:
PRINCIPAL HOSTS:
SPECIAL FEATURES:

Fig. 2.

Fig. 2 shows the characters included in the key to the Polyporaceae.

Subsidiary characters not printed on key for Polyporaceae:

a. Upper surface fawn
b. Upper surface ferruginous
c. Upper surface pallid
d. Upper surface sulcate
e. Upper surface sticky

The characters on which to base the key have been chosen as being those which can generally be determined with minimum delay, and the characteristics of the fungi when grown in culture on agar medium have not been included although in the case of some genera of Polyporaceae, e.g. *Poria* spp., they are very helpful. A similar card key for the identification of cultures of wood-rotting fungi is now in the course of preparation and will be published later if it proves useful. Only by using these keys will their flaws and omissions become apparent, and I shall be grateful for any suggestions or criticisms that occur to other workers.

SUMMARY

A method is described for the preliminary identification of Agaricaceae and Polyporaceae by the use of perforated cards. Specimen cards listing the features used for the diagnosis of species in these two families are illustrated.

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REFERENCES

CLARKE, S. H. (1938). A multiple-entry perforated-card key with special reference to the identification of hardwoods. *New Phytol.* CXXXVII, 370–4.
PEARSON, A. A. (1946). Notes on the Boleti. *Naturalist*, July–September.
PHILLIPS, E. W. J. (1941). The identification of coniferous woods by their microscopic structure. *J. Linn. Soc. Lond.* LII, 259–320.
RAMSBOTTOM, J. (1923). *A Handbook of the larger British Fungi.* London.

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DOWNY MILDEW DISEASE OF THE CULTIVATED LETTUCE

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A. Introduction

Mildew of lettuce, caused by *Bremia Lactucae* Regel, is a disease of long standing and one which occurs more or less in all places where lettuce is grown. It has been reported from all continents and from both temperate and tropical regions. The disease is readily recognized and is not likely to be confused with any others which infect the crop, and there has never been any doubt as to the identity of the causal organism. While, therefore, so much is definite, it is somewhat surprising that certain features of the disease are still largely not understood. Thus it is not clear by what means the fungus carries over from one crop to a succeeding one—in particular, it is not known whether the disease is transmitted by means of contaminated or infected seed or by infected residues of earlier crops. Morphologically the same species occurs as a parasite on various composite weeds (groundsel, *Sonchus*, etc.), and it is not certain how far these may function as intermediate hosts.

Again, though the disease is nearly always to be found without difficulty upon growing crops, there is little substantial evidence bearing on the damage which it produces. Affected plants, as a rule, are not killed, and they may in the long run mature satisfactorily. In the language of the cultivator, the plant often 'grows away from the disease', by which is meant that it does not die, but finally produces a saleable lettuce. It does not follow, however, that the effect of the disease is negligible—maturity may be delayed or the size of the mature plant may be reduced. Clear evidence on this point is lacking.

The work described in this paper was carried out over the two seasons 1939–40 and 1940–1 and relates mainly to the points raised in the foregoing paragraphs, viz. the mode of transmission of the disease, and the influence of the disease on the maturing of the crop. Fungicidal methods of control have also been examined. These problems have been studied under the conditions of cultivation which prevail in the market-garden area which lies in the Thames valley west of London, and as the mechanism of disease transmission is obviously related to the methods of cultivation practised, a short account of the latter is desirable.

B. Methods of cultivation

Beginning in September, winter lettuce is sown in the open, followed by a later sowing in frames from late October to early November, the latter crop being planted out in spring. These crops mature from about early

May to the middle of June. During approximately the same period, i.e. from September to April, lettuce crops may be grown and matured in frames (cold or heated) or as catch crops in greenhouses. Finally, summer lettuce is sown in the open from March onwards, and these crops mature from July to as late as December, if the early winter is not too severe.

Thus on any market garden in the area round London, there is much overlapping of lettuce crops. It is particularly noteworthy that winter lettuce, which constitutes a fair proportion of the new year's early field crop, overlaps by about two months the last of the preceding year's summer crop. The weather in October–December is often very favourable to mildew, and in fact this disease is often rampant in late summer crops. The new crop cannot always be segregated from such infected earlier crops and therefore there is the possibility of wind-borne spores passing from the one to the other.

My attention was directed chiefly to the incidence of mildew in 'Spring lettuce', i.e. lettuce which is sown in unheated frames in late October or early November and planted out from February to April. In such frames one frequently sees isolated seedlings with one or both cotyledons carrying mildew. These occur sporadically, though later whole areas of the frames may become affected. The first outbreak of the disease thus appears to suggest seed-borne transmission.

C. TRANSMISSION OF MILDEW BY SEED

The method used for investigating the possibility of mildew being seed-borne was as follows. Seed-boxes were dipped for five to ten minutes in boiling water and filled with soil which had been autoclaved for one and a half hours. In order to safeguard against air-borne contamination, the boxes after being seeded were covered with glass plates or bell-jars. In later experiments, an improved device, viz. a large glass-sided frame with closely fitting glass lid, was used. The boxes were freely watered with sterile water, and thus a highly humid atmosphere, favourable to the development of mildew, was provided.

From three to six weeks after sowing, according to the time of year, the seedlings had developed their first two foliage leaves. It could thus be assumed that any seed-borne infection would have had time to produce conidia on the cotyledons, and so the seedlings were removed singly with forceps and examined for the presence of mildew.

In the first five experiments of this kind Trocadero Improved, Imperial and Early French Frame seed of the current year (1939) was used. Later (Exps. VI–VIII) older seed of Trocadero was tested as the 1939 stock may have been unusually clean. The commercial seed so far used may, however, have been harvested from plants which were uninfected by mildew and so in the autumn of 1940 seed was harvested from Imperial, Trocadero, and Winter King plants, the first of which were known to have been heavily infected, but the other two had only been slightly infected and then not at the harvesting period. With the expectation that seed of Imperial mother plants so infected would be most likely to show whether seed-borne transmission of mildew does or does not occur, four experiments (XI–XIV) were carried out with this seed at various times over the winter of 1940–1.

The data obtained from the fourteen experiments bearing on the question of seed-borne transmission are assembled in Table 1. The results may be summarized as follows:

(1) Imperial, Trocadero and Early French Frame seed of 1939 showed no seed-borne transmission of the disease.

(2) One sowing of commercial Trocadero seed (Exp. VI) contained a small percentage of infected seedlings but the other two did not.

(3) Two experiments (XI and XII) with seed taken from infected mother plants gave slight infection but four did not.

(4) The total number of seedlings examined from October 1939 to May 1941 was 23,755 and of these only 0·025 % were infected with mildew.

Table 1. *The percentage seed infection of lettuce by mildew*

Exp.	Seed sown	Total seedlings	No. infected	% infected seedlings	Variety
I	1. x. 39	1055	0	0	1939 Troc.
II	15. xii. 39	1245	0	0	1939 Imp.
III	15. xii. 39	945	0	0	1939 E.F.F.
IV	15. xii. 39	813	0	0	1939 Troc.
V	25. i. 40	2045	0	0	1939 Imp.
VI	29. iii. 40	1784	3	0·16	1938 Troc.
VII	29. iii. 40	2141	0	0	1938 Troc.
VIII	20. v. 40	946	0	0	1936 Troc.
IX	24. ix. 40	1213	0	0	1940 W. King
X	24. ix. 40	1014	0	0	1940 Troc.
XI	24. ix. 40	699	1	0·14	1940 Imp.
XII	23. x. 40	4069	2	0·05	1940 Imp.
XIII	11. xii. 40	1403	0	0	1940 Imp.
XIV	10. iii. 41	4383	0	0	1940 Imp.

D. TRANSMISSION BY DEBRIS OF INFECTED CROPS

Lettuce frames are often set up in the same place as in the preceding years, and succeeding field crops sown in the same ground, because market gardeners in this area use intensive methods of cultivation and cannot practise a long-term rotation system. It is possible therefore that viable fungal residues may carry the disease over in the field or in frames, particularly in the latter. This brings into consideration the question of resting structures, in particular oospores, and of the viability of mildew conidia.

Oospores of *Bremia Lactucae* were first described by de Bary (1863) but from *Senecio vulgaris* only. References to their occurrence on the cultivated lettuce are given by Smith (1884), Schweizer (1919), Lavrov (1932), and Baudys (1935), and from these it is apparent that oospores are rarely found on that host.

An attempt was made to find them in the debris of lettuce plants which were heavily infected with mildew when alive. Lettuce leaves have a high-water content and a low proportion of lignified tissue, so that they rapidly disintegrate and disappear soon after falling to the ground. If, however, the soil is not too wet they become dry and after a few months in winter are black and friable. For microscopic examination this type of material was cleared by immersion in a mixture of equal parts of chloral hydrate and pure phenol crystals. These mix on being gently warmed, and on cooling the mixture remains liquid. The fragment of leaf was then placed in 10% caustic potash solution for a few minutes, washed in several changes of water, stained with aniline blue and finally cleared with lactic acid.

Many slides were prepared in this way from decayed lettuce leaves, but no oospores were seen. On the other hand, recognizable mycelium of *Bremia Lactucae* was almost invariably present, and it is possible that portions of this mycelium may long remain alive.

To test whether soil containing debris from infected plants could infect seedlings, a stock of soil was built up between December 1939 and May 1941 by the continued addition of badly infected leaves to a large box of soil. At intervals some of this soil was removed and about one-third of the sample autoclaved at 15 lb./sq.in. for 1½ hours to be used as control. Commercial seed was then sown in both lots of soil and protected from wind-borne spores as in the experiments described in the previous section. The varieties used were Trocadero and Imperial, the earlier tests having shown that the commercial seed of these varieties did not carry mildew. To encourage development of mildew the humidity was kept at a high level by watering freely with sterile water. After three or four weeks the seedlings were examined for infection, with the results shown in Table 2.

Thus, in all, over 5000 seedlings were grown under conditions highly favourable to the development of mildew, but only one infected seedling was seen. As much debris was present it seems that any viable fungal remains in it were ineffective in transmitting the disease.

Table 2. *Effect of contaminated soil on the occurrence of mildew in lettuce seedlings*

		Contaminated soil		Controls		
Exp.	Date of sowing	No. of plants	% infected	No. of plants	% infected	Variety
I	13. iii. 40	378	0	137	0	Troc.
II	13. iii. 40	859	0·1	256	0	Imp.
III	3. v. 40	2021	0	1562	0	Troc.
IV	8. v. 40	641	0	381	0	Imp.
V	22. x. 40	689	0	311	0	Imp.
VI	9. iv. 41	558	0	879	0	Imp.
	Total	5146	0·02	3526	0	

The possibility of a carry-over by conidia is unlikely but it was desirable to determine the time limit of their viability. For this purpose a number of slides were plentifully dusted with conidia, placed in Petri dishes and kept out of doors at a time when weather conditions were favourable to the development of mildew. On each successive day two slides were moistened with distilled water and twenty-four hours later these were examined for germination. The results from three experiments of this kind are given in Table 3, the figures being the average percentage germination of the conidia for each pair of slides.

Table 3. *Viability of mildew conidia*

	% germination after exposure for							
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days
Exp. I	53	56	51	7	4	0·8	0	0
Exp. II	68	63	65	13	1	0·7	0·6	0
Exp. III	72	65	62	15	3·5	1·1	0·5	0

Under the prevailing conditions, viability ceased after the sixth or seventh day. It is clear therefore that while conidia remain viable long enough to allow of their being widely dispersed by wind, they cannot transmit the disease over a lengthy time interval.

E. Transmission by weeds

Bremia Lactucae occurs on a large number of composite genera such as *Sonchus*, *Tragopogon*, *Crepis*, *Senecio* and *Hieracium*, and these include many common species of weeds which may provide a source of infection of the cultivated lettuce.

In the autumn of 1940 seeds were collected from the following composite weeds: *Sonchus oleraceus*, *Tragopogon*, *Hieracium Pilosella*, *H. murorum*, *Lactuca muralis*, *L. Scariola*, *L. saligna*, *Senecio Jacobaea* and *S. vulgaris*. Seeds of these were sown under glass in September 1940 and grown to the three- or four-leaf stage, after which they were sprayed with a suspension of *Bremia* spores from cultivated lettuce by means of an atomizer. *Lactuca saligna* and *L. Scariola* only developed mildew lesions. A repetition of the experiment gave the same result, and the infections were successfully transferred from the wild species of *Lactuca* back to healthy cultivated lettuces.

Later, mildew was found occurring naturally on *Senecio Jacobaea* and this was transferred to *S. vulgaris* seedlings but it failed to infect the cultivated lettuce. *Bremia* found on *Senecio vulgaris* in the field behaved in a similar way, i.e. it infected *S. Jacobaea* but not the cultivated lettuce.

Bremia, therefore, though morphologically uniform, has various biological races. These different races show some specific choice of a host, e.g. *Lactuca muralis* was not infected by conidia taken from *L. saligna*, *L. Scariola*, or the cultivated lettuce.

It appears therefore that, of the wild hosts examined, *L. Scariola* and *L. saligna* are the only ones which are capable of transmitting mildew to lettuce. As both these are uncommon in England, they cannot be of any importance in this connexion.

The conclusions to be drawn from sections C, D and E are on the whole negative and therefore the only plausible hypothesis is that each lettuce crop is infected from a previous one. The implications of this will be discussed later.

F. Importance of the disease

Repeated observations of mildewed plants show that they become more susceptible to secondary attacks of the more important *Botrytis* disease. Mildew lesions on the outer leaves of more or less fully grown lettuce plants become senescent and *Botrytis* invades the leaves from these areas. Usually, however, mildewed plants do not succumb, but mature in due course. Damage therefore is to be assessed by comparing the performance in the field of mildewed seedlings with that of uninfected seedlings of the same age and cultural treatment. This type of experiment would not have much practical significance if the grower were able to discard all infected seedlings when planting out, but mildew is often so widespread in the frame that selection is not possible, and plants with very small or incipient lesions could not be sorted out.

Eight plots of lettuce were planted out from Dutch lights on 28 February 1941, each plot consisting of four rows of fifteen plants. The odd and even plots consisted of mildew-free and mildewed seedlings respectively, and all the plants were, as far as could be seen, free from infection by *Botrytis* and *Rhizoctonia*. Care was taken to see that all the plants were of uniform size. The survival figures for each plot were noted on 30 April and 2 June 1941, and these are shown in Table 4.

Table 4. *Effect of mildew on survival of lettuce*

		Plants per plot		
Plot	Seedlings	28 Feb.	30 Apr.	2 June
I	No mildew	60	56	52
II	Mildewed	60	37	32
III	No mildew	60	47	40
IV	Mildewed	60	35	31
V	No mildew	60	44	42
VI	Mildewed	60	37	32
VII	No mildew	60	56	54
VIII	Mildewed	60	45	43
Totals	Free from mildew	240	203	188
	Mildewed	240	154	139

The presence of mildew thus causes a material increase in the mortality of the plants, due in this experiment as usual to *Botrytis cinerea*.

The cutting record of this crop is shown in Table 5.

Table 5. *Numbers of lettuce matured at various dates*

		No. of lettuce cut				
Plot	Seedlings	6 June	12 June	16 June	19 June	Residue
I	No mildew	31	8	10	1	2
II	Mildewed	11	6	10	3	2
III	No mildew	31	4	0	3	2
IV	Mildewed	9	8	7	5	2
V	No mildew	29	5	3	2	1
VI	Mildewed	9	10	6	7	1
VII	No mildew	37	7	4	3	2
VIII	Mildewed	21	12	3	5	0
Totals	Free from mildew	128	24	17	9	7
	Mildewed	50	36	26	20	5

By the method of linear interpolation, one can determine approximately the dates at which 75 % of the two lots of plants matured. These are 8 and 14 June for the plants from non-mildewed and mildewed seedlings respectively. This represents a check to maturing of about six days, which is an item of importance to the growers of spring lettuce.

G. CONTROL BY MEANS OF RESISTANT VARIETIES

Workers in several countries have claimed that lettuce shows varietal resistance to mildew. Jagger and Chandler (1932) demonstrated that immunity is a Mendelian dominant and named eight resistant varieties. According to Macpherson (1932) the English varieties Loos Tennis Ball, Rosy Spring, and May Queen are resistant.

For the purposes of testing, seedlings three weeks old and of comparable size were evenly spaced in pots. There were ten seedlings per pot and ten pots of each variety. They were then inoculated with mildew spores under identical conditions, as follows. A suspension of fresh spores was put in a type H 3 Aerograph spray gun, constant pressure provided by a hand pump supplying air at 15 lb./sq.in. and the nozzle pointed horizontally at the seedlings with its tip at a distance of 9 in. from the near edge of the pot. Each pot was sprayed for one second.

Table 6 gives the percentage of infected plants in an experiment with nine commonly grown varieties.

Table 6. *Varietal susceptibility to mildew*

Variety	% infection	Variety	% infection
Lees' Immense	1·1	Cheshunt Early Giant	20·0
Trocadero	2·5	Arctic King	21·3
Market Favourite	6·3	Blackpool	21·3
Feltham King	12·9	Early French Frame	40·0
Imperial	15·0		

On analysing the data obtained in this experiment by the method of Bliss (1937, 1938), the following conclusions were drawn:

(1) Lees' Immense is significantly more resistant than Feltham King and all other varieties below it in the table.

(2) All the varieties are significantly more resistant than Early French Frame.

(3) Trocadero and Market Favourite are significantly more resistant than all the remaining varieties except Feltham King and Imperial.

In experiments which will be described later, the varieties Trocadero and Imperial, which would be classed respectively as resistant and moderately resistant on the basis of the results in Table 6, gave over 90 % of infected plants. Thus it is seen that, although there is a measure of variation in resistance between varieties, all those used by the writer have shown heavy infection under conditions favourable to the development of mildew. The choice of resistant varieties is not likely therefore to eliminate infection by mildew.

H. EFFECT OF VENTILATION OF FRAMES

That high humidity favours the disease is unquestioned and this was borne out by my experience in inoculating lettuce seedlings with mildew throughout this work. Growers accept this view and accordingly give as much ventilation as the atmospheric conditions permit. Thus it is good practice, in the management of frames, to remove the covers completely on dry non-frosty days, and to allow a certain amount of ventilation at night, in all but severe weather, by propping up the covers. It is true that other considerations apply, e.g. this procedure is believed to give some protection against *Botrytis* disease. Also complete removal of lights whenever possible allows of better illumination, so that hardier, stockier, more frost-resistant seedlings are produced.

Though so much is accepted, there is little quantitative evidence that humidity influences the spread of mildew; consequently the following experiment was devised. Two pots of similar size, each containing ten seedlings heavily infected with mildew were placed in the centre of neighbouring frames. Twenty pots, each containing ten healthy, three-week Imperial seedlings were placed round these pots at a distance of 2 ft. Both frames were then kept shut and one watered every day, the other not at all. After twenty-three days mildew lesions were noticed and the percentage infection determined. That in the watered frame was 61·4 % and that in the unwatered frame was 11·3 %. Between 27 March and 9 April 1941 the experiment was repeated and the results were:

Watered frame	38·6 % infection
Unwatered frame	0·0 % infection

These results are very clear-cut but though the humidity of the watered frame was certainly higher than that of the dry one some of the spread of the disease may have been due to the draughts and splashing caused by watering.

Whether the opening of the frames be good or bad from the point of view of draughts, it is certain that during spells of very humid, muggy weather opening of the frames cannot reduce the humidity to the safety level. The fact that mildew does appear in quantity both in frames and out of doors, especially in mild wet winters, is evidence that the ventilation method, even if it is useful, does not give adequate control under conditions which are highly favourable to the disease.

I. Fungicidal control

Experiments on this subject were carried out over the period November 1939 to April 1941. The general plan was as follows. For each treatment ten pots were set up, each containing ten three-week seedlings. These were hardened off and then sprayed or dusted with the appropriate fungicide. Next day they were inoculated with a suspension of *Bremia* conidia. The inoculum was prepared in tap water by brushing off spores from fresh mildew lesions with a wet camel-hair brush. Examination of a drop under the microscope showed whether it was concentrated enough, and usually it was filtered through butter muslin to remove conidiophores and dirt.

In 1939–40 the inoculations and sprays were applied with a small scent-spray atomizer but in 1940–1 a type HS Aerograph spray gun with a de Vilbiss nozzle was substituted since it gave a more constant and finely divided spray. The air pressure of 15–20 lb./sq.in. required for the operation of the atomizer or spray gun was provided by a small hand pump. Dusts were applied with an apparatus specially constructed to give an even flow of dust into a chamber of known area containing the pots to be dusted.

The pots of seedlings, after treatment with fungicide and spraying with spores, were randomized in a cold frame which was kept closed. After some days, varying in number with the temperature, but usually about twelve, conidia had developed on the leaves. Each seedling, which had by this time at least four leaves including the cotyledons, was examined and the number of infected and uninfected leaves noted. At the same time the amount of phytocidal damage was assessed for each pot according to an arbitrary scale, the figures 0, 1, 2, 3 corresponding respectively to 'no damage', 'just visible flecking', 'fairly general spotting' and 'development of considerable necrotic areas'. The highest figure obtainable in any experiment of ten pots was therefore thirty. Records of maximum and minimum temperatures were kept throughout the experiments.

Experiment I, begun on 12 November 1939, contained nine treatments:

(1) 5 % red copper oxide with talc as filler and applied as a dust at the rate of 0·25 oz./sq.yd.

(2) 5 % red copper oxide with Folosan (pentachloronitrobenzene) as filler, 0·25 oz./sq.yd.

(3) ½ lb. red copper oxide, ½ gal. cotton-seed oil, and 1 quart of lethalate spreader made up in 50 gal. of water.

(4) 2 : 2 : 40 Bordeaux mixture as a spray.

(5) Folosan as a dust at 0·25 oz./sq.yd.

(6) Folosan with lime as filler at 0·25 oz./sq.yd.

(7) Flowers of sulphur as a dust at 0·25 oz./sq.yd.

(8) Pure calcium hydroxide dust at 0·25 oz./sq.yd.

(9) Control.

Bordeaux mixture was included because of its successful use at this strength for the control of mildew by Osmun (1934), the copper oxide sprays and dusts because of the work done by Clayton (1938*b*) in the control of downy mildew of tobacco, and the Folosan because of its control of *Botrytis* disease of lettuce as demonstrated by Smieton and Brown (1940). Lime was included because some growers use it in the hope of reducing mildew.

The results of this experiment are given in Table 7 and may be summarized as follows:

(1) Lime is of no value.

(2) Sulphur, though it causes no injury, gives poor protection.

(3) Folosan, with talc filler, gives fair control and is safe; on the other hand, the incorporation of lime as filler more or less completely takes away its fungicidal value.

(4) All the preparations containing copper give a pronounced measure of control, but those containing copper oxide (Cu_2O) have rather a pronounced phytocidal effect. The best control is given by 2 : 2 : 40 Bordeaux mixture.

Table 7. *Effect of fungicides on the control of lettuce mildew*

Treatment	Plants		Leaves		Estimated damage
	Total	% infected	Total	% infected	
(1) Copper oxide + talc	192	0·0	746	0·0	14
(2) Copper oxide + Folosan	197	1·5	795	0·4	10
(3) Copper oxide spray	232	1·7	887	0·5	9
(4) 2 : 2 : 40 Bordeaux	205	2·5	794	0·8	2
(5) Folosan	207	13·1	804	5·2	0
(6) Folosan lime	214	78·0	803	36·4	0
(7) Lime dust	274	92·7	1018	57·9	0
(8) Sulphur dust	224	40·6	841	19·7	0
(9) Controls	213	87·9	789	49·8	0

Average minimum temperature = 41·0° F.

Experiment II. This was essentially a repetition, in duplicate frames, of the preceding. It was begun on 19 December 1939, but was not concluded until 25 January 1940 on account of the very slow development of mildew. During this period the exceptionally cold spell of the winter of 1939–40 began. This led to considerable winter-killing by frost, in spite of the protection afforded by the frames. The data of Table 8 show that this effect was accentuated by the use of fungicides, and in particular by certain of the copper-containing preparations. Of these, dilute Bordeaux mixture was, as in Table 7, the least damaging.

Table 8. *Mortality of lettuce seedlings due to frost*

Treatment	Exp. A % mortality	Exp. B % mortality
(1) Copper oxide + talc	55	50
(2) Copper oxide + Folosan	65	65
(3) Copper oxide spray	69	55
(4) 2 : 2 : 40 Bordeaux	40	42
(5) Folosan + talc	48	50
(6) Folosan + lime	42	41
(7) Lime	39	29
(8) Sulphur	31	30
(9) Control	37	31
Av. min. temp.	26·5° F.	25·2° F.

In further experiments during 1940–1 sprays only were used, and the list of these is as follows:

(1) *Cuprous oxide.* 'Cuprocide 54', a commercial preparation containing 50 % Cu_2O with incorporated spreader, was used at strengths of 2, 1, 0·5 g./l.

(2) *Bordeaux mixture* of compositions 1 : 1 : 40, 2 : 2 : 40 and 4 : 4 : 40.

(3) *Excess-Lime Bordeaux* (2 : 10 : 40). The fungicidal efficiency of this preparation has been demonstrated by Grubb (1924).

(4) *Sodium orthophosphate-copper sulphate spray.* Bruno (1940) gives the formula for a copper fungicide as: 1 kg. copper sulphate in 50 l. of water, and 1·25–1·30 kg. sodium orthophosphate in 20–30 l. of water, made up to 100 l. in water. He claims that it is more dispersible than Bordeaux mixture. This spray contains approximately the amount of copper present in a 4 : 4 : 40 Bordeaux mixture. For use on lettuce it was reduced to half the strength advised by Bruno to make it comparable with a 2 : 2 : 40 Bordeaux mixture. Sodium orthophosphate, unlike lime, dissolves completely in water, and the precipitate formed when copper sulphate is added

is finely divided and flocculent. This fungicide therefore gives a more even dispersion of small particles over the leaf surface than does Bordeaux mixture.

(5) *Bouisol* at the rate of 1 lb. to 30 gal. of water. At first the spreader Agral 2 (0·05 %) was added to the Bouisol but was later left out as it was found to increase damage.

(6) *Salicylanilide* at the rate of 10 oz./40 gal. of water with 0·05 % Agral 2 as a spreader.

Table 9

Experiment	I		II		III		IV		V	
Date: From	13. x. 40		16. x. 40		5. xi. 40		28. xi. 40		28. xi. 40	
To	31. x. 40		1. xi. 40		19. xi. 40		9. i. 41		17. i. 41	
Average min. temp. ° F.	34·0		36·0		35·1		29·5		31·0	
Fungicide	E.D.	% inf.	E.D.	% inf.	E.D.	% inf.	E.D.	% inf.	E.D.	% inf.
¼ strength Cuprocide	0	20·6	—	—	—	—	—	—	—	—
½ strength Cuprocide	1	13	0	18·1	0	2	—	—	3·5	0
Full strength Cuprocide	3	21·8	1	18·7	2	10	7·5	2	6	0
1 : 1 : 40 Bordeaux mixture	2	7·1	0	2·1	2	1·2	18	2	11	0
2 : 2 : 40 Bordeaux mixture	5	1	2	1	5	0	24	1·1	12	0
4 : 4 : 40 Bordeaux mixture	5	1·2	3	0	6	0	21	0	14	0
Excess-Lime Bordeaux	—	—	—	—	—	—	10·5	2	—	—
Orthophosphate-copper spray	—	—	—	—	—	—	—	—	—	—
Bouisol	—	—	—	—	—	—	—	—	—	—
Bouisol + Agral 2	—	—	—	—	6	0	22	0	23	0
Salicylanilide + Agral 2	—	—	0	40·8	—	—	—	—	—	—
Control	0	53	0	46·8	0	4	4	53·1	0	10

Experiment	VI		VII		VIII		IX	
Date: From	24. i. 41		28. ii. 41		21. iii. 41		9. iv. 41	
To	25. ii. 41		18. iii. 41		4. iv. 41		23. iv. 41	
Average min. temp. ° F.	34·0		35·2		37·9		39·0	
Fungicide	E.D.	% inf.	E.D.	% inf.	E.D.	% inf.	E.D.	% inf.
¼ strength Cuprocide	—	—	—	—	—	—	—	—
½ strength Cuprocide	—	—	—	—	—	—	—	—
Full strength Cuprocide	2	0	0	0	0	1	0	12
1 : 1 : 40 Bordeaux mixture	4·5	0	0·5	0	1·5	2	0	15
2 : 2 : 40 Bordeaux mixture	3·5	0	0	2	3·5	0	0	16
4 : 4 : 40 Bordeaux mixture	4	1	0·5	1·1	1·5	5	0	18
Excess-Lime Bordeaux	0	1	0	2	1	1	—	—
Orthophosphate-copper spray	0·5	1	0	1	0	0	0	13
Bouisol	3	0	0	0	3·5	0	0·5	12
Bouisol + Agral 2	2·5	0	—	—	—	—	—	—
Salicylanilide + Agral 2	—	—	—	—	—	—	—	—
Control	0	33·8	0	61·9	0	72·6	0	96

E.D. estimated damage.

Table 9 shows the relation between fungicidal efficiency, damage, and average minimum temperature. The following are the conclusions from this table.

(1) The amount of damage increases as the average minimum temperature falls.

(2) The damage varies with the fungicide.

(3) As the strength of the Bordeaux increases there is no marked increase either in fungicidal effect or phytocidal damage though on the whole 2 : 2 : 40 Bordeaux gives the best control.

(4) Cuprocide 54 is somewhat safer at low temperatures than Bordeaux 2 : 2 : 40 and in addition it is just as effective as a fungicide.

(5) Bouisol at the strength used (1 lb. to 30 gal. of water) has no advantage over Bordeaux as a control and causes more damage.

(6) 2 : 10 : 40 Excess-Lime Bordeaux causes less damage than 2 : 2 : 40 Bordeaux mixture but its efficiency as a fungicide may be a little less.

(7) The sodium orthophosphate-copper sulphate spray, over the short period during which it was used, proved as efficient as 2 : 2 : 40 Bordeaux and also caused less damage.

(8) Salicylanilide + Agral 2 (5 %) gives a poor control of mildew.

The experiments so far described were carried out under conditions which were highly favourable to the development of mildew. In the course of the work three experiments under ordinary conditions of practice, except that the plants were uniformly inoculated at a young stage by spraying with spores, gave the results which are summarized below.

Experiment 1, with fifteen Dutch lights each containing forty-eight Imperial seedlings (23 Feb.–3 April 1940). Treatments: Control; Cuprocide 54 (½ lb. to 50 gal. water); Bordeaux mixture 1 : 1 : 40, 2 : 2 : 40 (with or without Folosan), 4 : 4 : 40. Two sprayings were applied. All treatments gave good control of mildew, the controls showing considerable infection in the young stages. The experiment was concluded when the plants were about half-grown, at which time there was no evidence of difference between controls and treatment in respect of numbers of survivors or weight of plants. This was apart from some check to growth where Folosan had been used.

Experiment 2, with six Dutch lights (4 Nov. 1940–13 May 1941). Treatments: Control; Bordeaux mixture 2 : 2 : 40 and 4 : 4 : 40. Three sprayings were applied. Conditions in early winter were not favourable to severe infestation of the controls. At the time of maturity there was no difference in any respect between treated and untreated plants.

Experiment 3, with sixteen Dutch lights (17 Dec. 1940–12 June 1941). Treatments: Control; Bordeaux 2 : 2 : 40; Cuprocide 54 (1 lb. to 50 gal. water); Bouisol (1 lb. to 30 gal. water). Two sprayings were applied. By mid-February heavy infestation of the untreated frames was shown, the treatments and especially 2 : 2 : 40 Bordeaux showing good control. Seventy-two plants from each treatment were set out in replicated plots in the open ground on 18 Feb. and carried to maturity. The differences in survival and in average mature weight were negligible, but there was a delay of five days in the maturing of the untreated plants. This amount may be of significance to the practical grower, at least at certain seasons.

Attempts to use the vapour method which has proved successful in the control of tobacco mildew (blue mould), viz. to use paradichlorbenzene as recommended by Clayton (1938*a*, *b*) or benzene mixed with lubricating oil as recommended by Wolf *et al.* (1939, 1940) have given very unsatisfactory control of lettuce mildew and do not appear at all promising.

J. EFFECT OF *p*H ON GERMINATION OF *BREMIA* CONIDIA

The preliminary experiments in the section on fungicidal control include hydrated lime, as lettuce growers consider that it gives some control of lettuce mildew. The results obtained show that lime is of no value whatever as a fungicide in this instance, and infection on lime-dusted plants is at least as heavy as on controls, if not heavier, the comparative percentage infections being:

Lime, 92·7 %, 98 %. Control, 87·9 %, 93 %.

Laboratory experiments on the germination of *Bremia* spores on lime-dusted slides showed that, as expected, lime dust had no effect on the germination. Both controls and dusted slides had germinations varying between seventy and eighty per cent. This shows that *Bremia* conidia are able to germinate in alkaline solutions. The effect of *p*H on germination is shown more fully in Table 10.

Table 10. *Effect of* *p*H *on the germination of conidia*

	% germination	
*p*H	Exp. A	Exp. B
9·6	73	65
8·0	67	76
6·4	53	62
3·9	22	34
2·2	12	5

Both experiments agree in showing that germination is reduced in distinctly acid solutions but takes place freely in alkaline solutions. The *p*H of saturated lime solution however is greater than 9·6 and so is outside their range. Titration with hydrochloric acid by the standard method gave the normality of saturated lime solution as *N*/34. Further experiments showed that germination falls off sharply beyond this point and that it is inhibited in *N*/10 sodium hydroxide. On the acid side germination begins to be affected in concentrations of HCl exceeding 1 in 10,000 *N*.

K. SUMMARY

1. Lettuce seedlings, grown from both ordinary commercial seed and seed from mother plants known to be infected with mildew, almost invariably failed to show symptoms of the disease under conditions favourable to mildew development. There was thus negligible evidence of the seed-borne transmission of mildew.

2. Oospores were not found by the microscopical examination of debris from infected plants, and seedlings grown in soil containing a high proportion of such debris for a period considered to be adequate, under favourable conditions for the development of mildew, did not become infected. The transmission of the disease by contaminated soil is therefore unlikely.

3. Attempts to transmit mildew from composite weeds to lettuce were successful only with some wild species of *Lactuca*. These species are probably too rare to be important in the spread of the disease.

4. The survival of mildewed lettuce was lower, and their date of maturity later, than that of healthy plants. This, together with the disfigurement caused by the disease, gives it its commercial importance.

5. A number of commercial lettuce varieties commonly grown in England showed varietal differences in their susceptibility to the pathogen but these differences were not sufficiently marked to make the exclusive cultivation of the more resistant varieties worth while as a control measure.

6. 2 : 2 : 40 Bordeaux mixture, Excess-Lime Bordeaux (2 : 10 : 40), an orthophosphate copper spray, and Cuprocide 54 (1 lb. to 50 gal.) all gave adequate protection from mildew disease but sprays containing no copper such as lime sulphur and pentachloronitrobenzene did not. All the copper-containing sprays are liable to produce phytocidal damage, particularly in cold weather.

7. Fungicidal vapours such as benzol and paradichlorbenzene were troublesome to use and gave a poor control of lettuce mildew.

8. *Bremia* conidia under favourable temperature conditions germinated readily in saturated lime solution and were also found to germinate more readily in slightly alkaline than in slightly acid solution.

I wish to thank Prof. W. Brown, F.R.S., who suggested this investigation and under whose supervision it was carried out.

REFERENCES

BAUDYS, E. (1935). Lettuce or salad mould. *Leafl. phytopath. Sect. Reg. Agric. Exp. Sta., Brno*, XCIII, 2 pp.

BLISS, C. (1937). The analysis of field experimental data expressed in percentages. *Plant Protection*, XII, 67–77.

BLISS, C. (1938). The transformation of percentages for use in the analysis of variance. *Ohio J. Sci.* XXXVIII, 9–12.

BRUNO, A. (1940). A new formula for a copper mixture. *C.R. Acad. Agric. Fr.* XXVI, 454–7.

CLAYTON, E. E. (1938*a*). Paradichlorbenzene as a control of blue mould of tobacco. *Science*, N.S. LXXXVIII, 2272, p. 56.

CLAYTON, E. E. *et al.* (1938*b*). Control of the blue mould (downy mildew) of tobacco by spraying. *Tech. Bull. U.S. Dep. Agric.* 650, 23 pp.

DE BARY, A. (1863). Recherches sur le développement de quelques champignons parasites. *Ann. Sci. Nat. Bot.* (Sér. 4), XX, 5–148.

GRUBB, N. H. (1924). Tests of fungicides on apple trees. *J. Pomol. Hort. Sci.* III, 157–62.

JAGGER, I. C. & CHANDLER, N. (1932). Physiologic forms of *Bremia Lactucae* Regel. *Phytopath.* XXIII, 18–19.

LAVROV, N. N. (1932). Key to plant parasites of cultivated plants of Siberia. Pt. 1. Abs. in *Rev. Appl. Mycol.* (1933), XII, 306–7.

MACPHERSON, N. J. (1932). The cultivation of lettuce under glass with special reference to varietal resistance to downy mildew. *J. Minist. Agric.* XXXVIII, 998–1003.

OSMUN, A. V. (1934). *Rep. Dep. Bot. Mass. Agric. Exp. Sta.* pp. 23–7.

SCHWEIZER, J. (1919). Die kleinen Arten bei *Bremia Lactucae* Regel. *Verh. Thuring. naturf. Ges.* XXIII, 17–61.

SMIETON, M. J. & BROWN, W. (1940). *Botrytis* disease of lettuce, its relation to damping-off and mildew, and its control by pentachloronitrobenzene dust. *Ann. appl. Biol.* XXVII, 489–501.

SMITH, W. G. (1884). Resting spores of *Peronospora gangliformis* Berk. *Gard. Chron.* XXI, 418.

WOLF, F. A. *et al.* (1939). Field studies on the concentration of benzol vapour as used to control downy mildew of tobacco. *Phytopath.* XXIX, 177–87.

WOLF, F. A. *et al.* (1940). Volatile fungicides. *Phytopath.* XXX, 213–27.

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A *VERTICILLIUM* DISEASE OF CULTIVATED MUSHROOMS NEW TO GREAT BRITAIN

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A new species of *Verticillium* attacking the brown strain of the cultivated mushroom, *Psalliota* (*Agaricus*) *campestris* (L.) Fr., was recently described in Copenhagen by Treschow (1941), who named it *Verticillium Psalliotae*. The same species was noticed at Yaxley in January 1946, attacking the white strain of the cultivated mushroom; and other specimens have since been seen at Thornham, Norfolk. On 29 January 1946, during the course of routine examinations of diseased mushrooms which modern commercial practice demands (Atkins, 1945), one specimen with a light brown, roughly circular patch on the pileus was found, as expected, to be attacked by a *Verticillium*, but the species differed radically from *V. Malthousei* Ware (1933) in that the conidia were invariably pointed at each end, and they were borne transversally on the phialides. Commercial practice compelled the removal of the diseased mushroom from the bed, but cultures were made in the laboratory. Three more similarly infected mushrooms were found in succeeding weeks, and detailed examination by Mr McG. Bulloch and myself revealed close similarity to *V. Psalliotae*, which Treschow found in the summer of 1939.

Treschow (1941) stated that the pathogenicity of both *V. Malthousei* and *V. Psalliotae* was confined to the brown strain. This contention is certainly invalid with regard to *V. Malthousei*, as Ware (1933) has demonstrated; and from the appearances at Yaxley it appears that the white strain is also attacked by *V. Psalliotae*. We sent cultures of our fungus to Dr Treschow, and after a preliminary examination he tells me he is unable to find any difference between it and *V. Psalliotae*. He found that the appearance of the strain, the position of the branches, and the size, as well as the characteristic red colouring matter formed in the substratum, were identical.

White mushrooms at Yaxley have been successfully inoculated with the disease by applying drops from a washing in sterile water of a malt agar slope culture. Vigorous growth of mycelium was visible to the naked eye after forty-eight hours at 20° C.

The disease is now being investigated further by Dr C. J. LaTouche, Microbiologist with the Mushroom Research Association at Yaxley. Preliminary observations reveal that the mould, when grown on 2 % malt agar (*p*H 5·5) at fluctuating laboratory temperatures, produced an abundant floccose snow-white aerial mycelium which was compacted to form a thin skin at the surface of the medium. It developed a pigment (Van Dyke Red, Ridgway) in the medium, as Treschow found. Aerial mycelium from a three-weeks-old culture on malt agar, mounted in lactophenol and cotton blue, revealed the following characteristics:

Hyphae septate, branched, about 1 μ broad. Fertile hyphae bearing more than four verticils of phialides, which in turn vary in number from two to five, measure 12·5–31·5 μ long by a maximum of 1 μ broad, and taper to a fine point. Conidia aseptate, more or less straight on the attached side and somewhat convex on the other, pointed at both ends, 4·3–12·9 μ × 1·75–2·15 μ broad (compared with Treschow's measurements of 6–10·5 μ × 2–3·5 μ).

REFERENCES

Atkins, F. C. (1945). *Verticillium* on mushrooms. *Mushroom Growers' Association, Midlands Group Publication, Yaxley, Peterborough*, pp. 9–25.

Treschow, C. (1941). The *Verticillium* diseases of cultivated mushrooms. *Dansk Bot. Ark.* xi, 1–31.

Ware, W. M. (1933). A disease of cultivated mushrooms caused by *Verticillium Malthousei* sp.nov. *Ann. Bot., Lond.*, xlvii, 763–85.

(*Accepted for publication* 31 *December* 1946)

STUDIES ON BRITISH CHYTRIDS

III. *ZYGORHIZIDIUM WILLEI* LÖWENTHAL AND *RHIZOPHIDIUM COLUMNARIS* N.SP.

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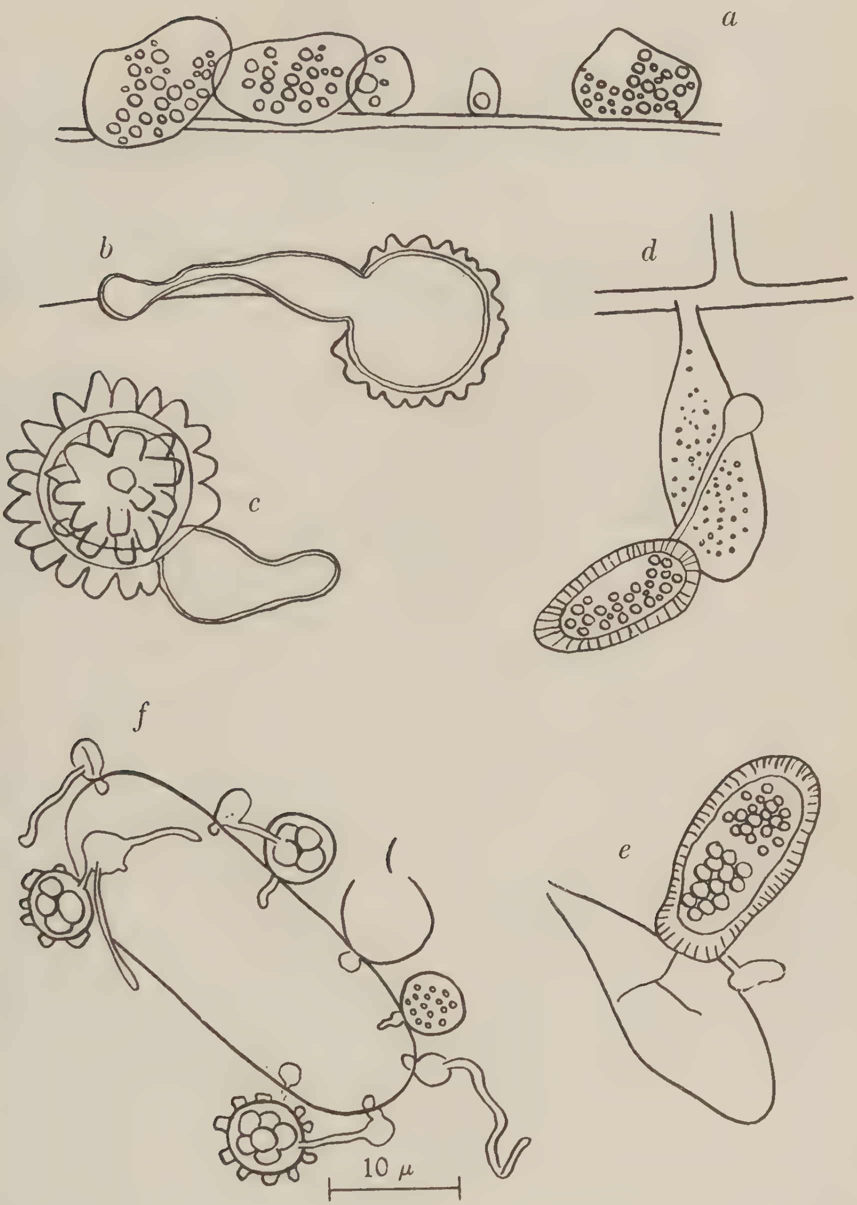
(With Plate XI and 4 Text-figures)

The genus *Zygorhizidium* was erected by Löwenthal (1905) for an epibiotic operculate chytrid, distinguished from *Chytridium* by possessing a resting spore formed as the result of a sexual process. The type of sexual reproduction exhibited by *Zygorhizidium* is well known. A small male thallus puts out a conjugation tube which grows until it makes lateral contact with the wall of a larger female thallus. Fusion occurs and the contents of the male pass into the female, which later becomes the resting spore. In addition to Löwenthal's observations on *Z. Willei*, it has been described by Scherffel (1925) and Domján (1936). *Z. verrucosum* Geitler (1942) has an identical sexual process (Text-fig. 1*f*).

This type of sexual reproduction has again been observed in *Chytridium Characii* Scherffel (1925). The mature resting spore is ovoid, with its long axis at right angles to the host cell. The outer layer of the wall is thicker at the base and apex than in the middle region, and in surface view is covered by longitudinal rows of elongate warts (Text-fig. 1 *d*, *e*). Although the method of formation of this resting spore is so strikingly like that of *Zygorhizidium*, the exact position of *Chytridium Characii* remains obscure since no zoosporangia were observed. The conjugation tube in the three organisms mentioned above is characterized by remaining equally cylindrical throughout its length. Scherffel (1925) describes *Chytridium*? *Spirotaenia*, in which the conjugation tube swells at its point of contact with the female, and becomes club-shaped (Text-fig. 1 *b*). Thus when the male and female thalli are close together, the narrow middle portion is lost, and the conjugation tube forms an irregular vesicular structure (Text-fig. 1 *c*). Once again the exact affinities of this organism are unknown, since dehiscence of the zoosporangium was never seen. The structure of the resting spore wall, with its outer surface covered with spines, consisting of refractive wall material, somewhat resembles *Zygorhizidium verrucosum*, and the type of sexuality may be regarded as a variant of that found in *Zygorhizidium*.

A few examples of *Z. Willei*, hitherto unknown from this country, were found growing on *Mougeotia* sp. in a collection from Montreal Park Lake, Sevenoaks, Kent, England, in March 1945. Little doubt remains as to the identity of this fungus although dehiscence of the zoosporangium was not



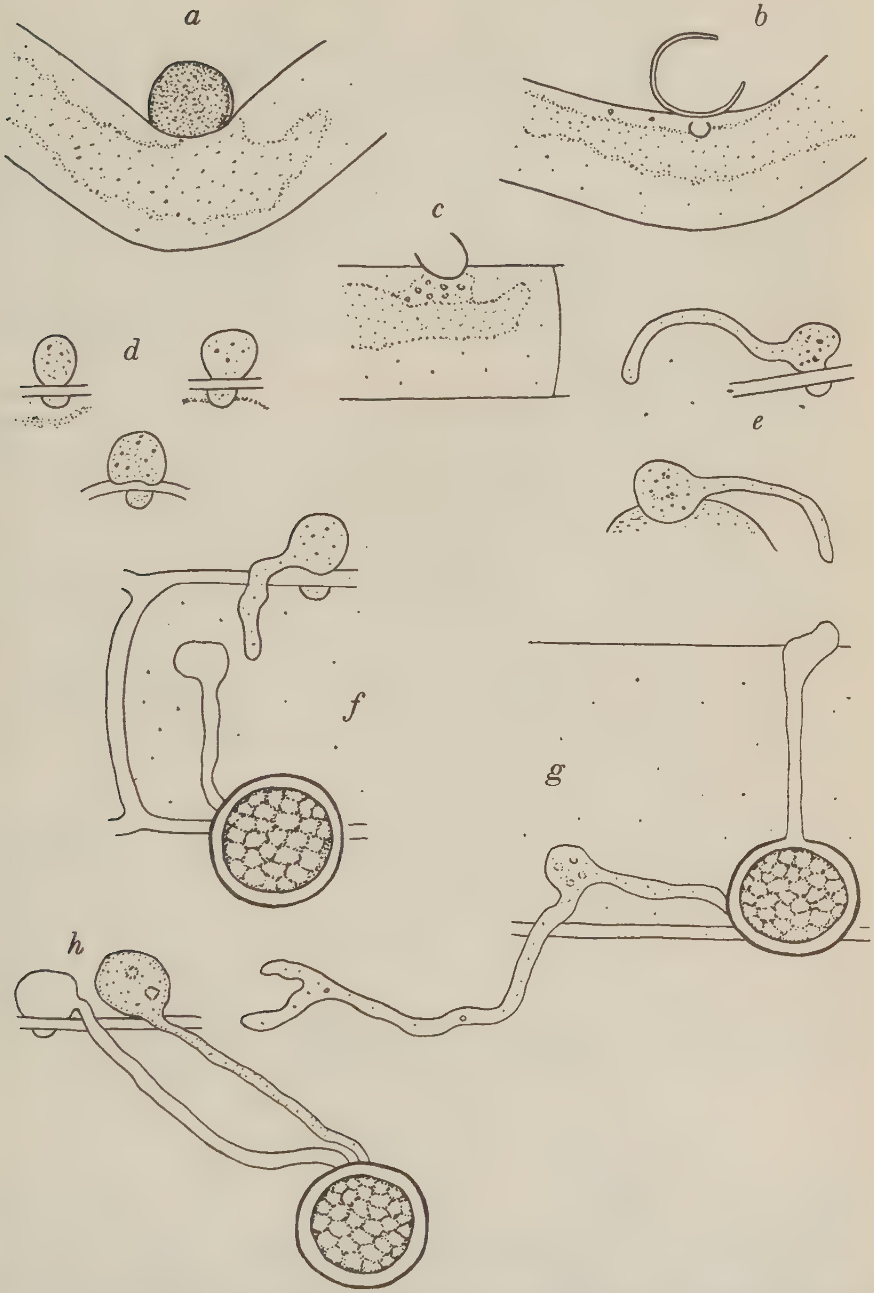
Text-fig. 1. *a–c*, *Chytridium Spirotaeniae* Scherffel. *a*, zoosporangia; *b*, *c*, sexually formed resting spores; in *c*, the middle cylindrical portion of the conjugation tube is lost, it therefore forms a swollen vesicular structure; *d*, *e*, resting spores of *Chytridium Characii* Scherffel; *f*, *Zygorhizidium verrucosum* Geitler. (*a–e*, after Scherffel (1925); *f*, after Geitler (1942).)

seen, since the shape of the sporangia and the peculiar method of sexual reproduction agree perfectly with Löwenthal's original description of *Zygorhizidium Willei* on *Cylindrocystis Brebissonii*, from Norway.

The fungus is parasitic, but rarely produces more than a slight disorganization of the host chloroplast. Large sporangia 17 μ in diameter may bring about a curvature of the *Mougeotia* cell as reported previously by Scherffel (1925), but this is not a constant feature, and dwarf sporangia (5–6·6 μ in diameter) caused no distortion of the filament. The intramatrical rhizoidal system consists of a knob-like structure apparently devoid of rhizoids (Text-fig. 2*b*). In some specimens it seemed to be absent, but more probably it was merely obscured by the chloroplast of the host cell. Zoospores were observed only once, and were of the usual chytridiaceous type.

After the material had been left in the laboratory for about four weeks, sexually formed resting spores appeared (Text-fig. 2*f–h*). The male thallus (Pl. XI, fig. 2), is ovoid and possesses an endobiotic knob devoid of rhizoids. The conjugation tube from the male thallus often branches, and may reach a length of 33 μ. Contact is made with a larger subspherical receptive thallus whose endobiotic knob also lacks rhizoids. In several instances two conjugation tubes approached the receptive thallus (Text-fig. 2*h*), but the one which had fused with it could be detected by its lack of contents. The resting spore is spherical, 11 μ in diameter, with a thick smooth wall, brownish in colour, surrounding the central highly refractive globular contents; germination was not observed.

In late August 1946 a chytrid, at first believed to be *Chytridium? Spirotaenia* Scherffel, was found parasitizing *Spirotaenia condensata* Brèb. in the Clay Pond, Wray Castle. The fungus appears to be specific to its host, since other Conjugales, *Mougeotia*, *Zygnema* and *Closterium* spp., although present, were not attacked. The epibiotic sporangium is usually broadly ovoid, very rarely spherical, with its longer axis parallel to the algal wall (Text-fig. 3*b–f*). A small conical protuberance, probably representing an unexpanded portion of the original zoospore case, can usually be found on the upper surface of each sporangium (Text-fig. 3*c*). The contents of the latter are in young stages mainly centralized, with a few scattered globules in the hyaline peripheral region. Later the content becomes evenly granular, and the subsequent changes in the protoplasm leading to the formation of zoospores are similar to those described for the majority of chytrids. The rhizoidal system where visible is not extensive and consists of a tuft of branched structures arising close together, so that no distinct main rhizoidal axis is produced (Text-fig. 3*c*, *d*). The mature sporangia vary from 25 to 63 μ broad × 16 to 27 μ high; a few extremely small ones rather more spherical were encountered 8–15 μ broad × 9–13 μ high. The number of zoospores produced in a mature sporangium varies according to its size. A small one liberates about fifteen zoospores, whereas up to one hundred are formed in a large sporangium. The sporangium wall gradually deliquesces, and two oppositely placed, broad dehiscence pores appear (Text-fig. 3*e*, *f*). The uniflagellate zoospores emerge singly, and swim away with a smooth gliding movement. They are spherical, 2·6 μ in



Text-fig. 2. *Zygorhizidium Willei*. *a*, immature sporangium on *Mougeotia*. *b*, dehisced sporangium with indications of a knob-like rhizoid. *c*, an empty small sporangium. *d*, young female thalli. *e*, developing male thalli. *f–h*, mature resting spores; in *h*, two male thalli are connected with one resting spore. (*a–c*, ×660; *d–h*, ×1400.)

diameter, with a conspicuous anterior oil globule and a darker area to one side of it.

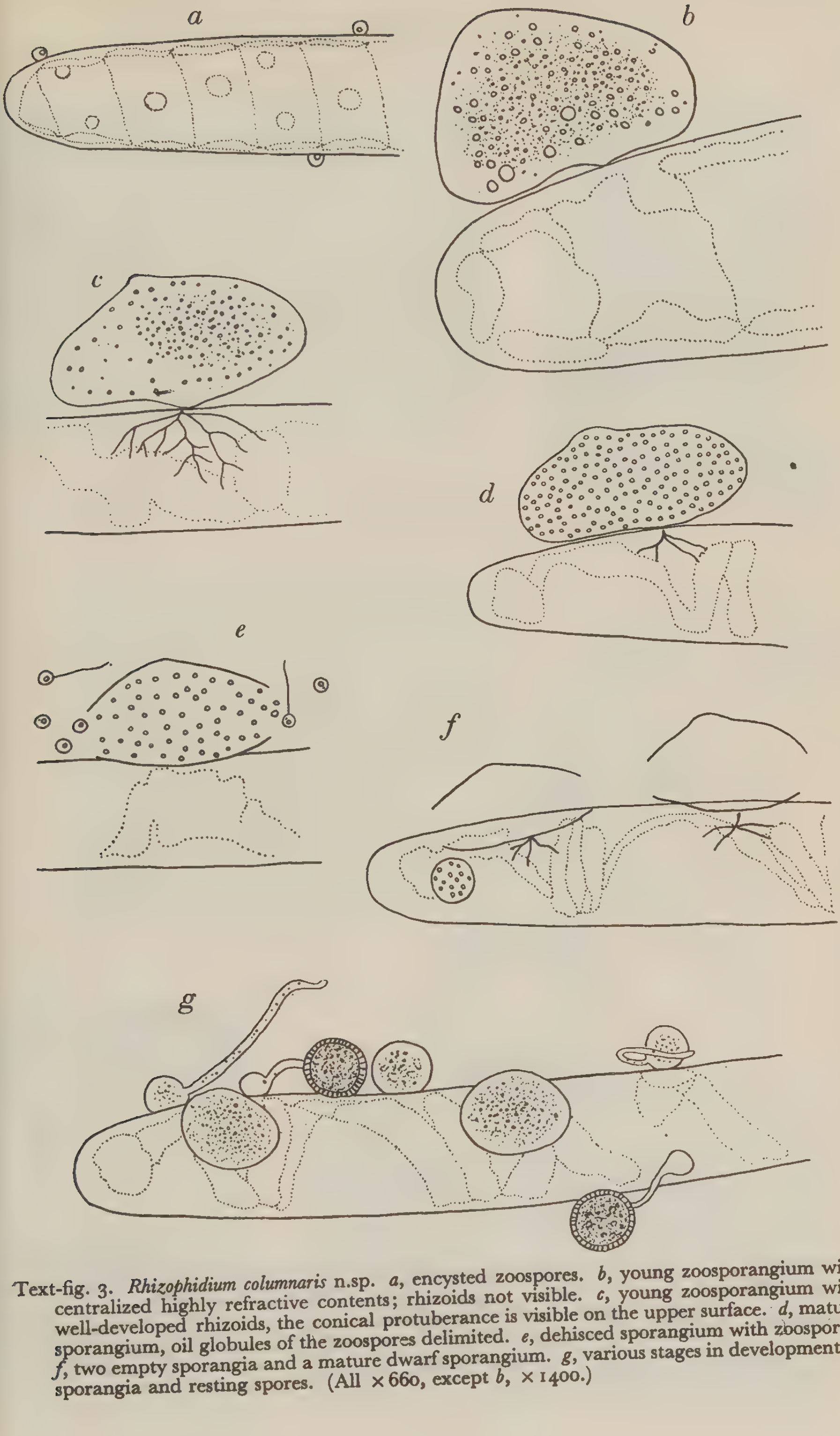
The resting spore is produced after a sexual process identical with that found in *Zygorhizidium*. The spherical male thallus puts out a narrow conjugation tube about 2 μ in diameter, up to 38 μ long, which grows until it reaches a female thallus (Text-figs. 3*g*, 4*h–k*, *n* and Pl. XI, fig. 5). The latter is also spherical, slightly larger than the male and having a broader base. The rhizoids of these thalli can rarely be distinguished. Presumably, following fusion, the wall of the female thickens (up to 3 μ diameter) and columnar bands of highly refractive wall material develop. These extra thickening bands are internal to the spore wall, which retains its smooth outline (Text-figs. 3*g*, 4*h*, *n* and Pl. XI, fig. 4). The mature resting spore is spherical, 10–20 μ in diameter, with granular contents; its germination was not observed.

As mentioned earlier (p. 130) this chytrid shows a striking resemblance to *Chytridium Spirotaenia*. The shape of the zoosporangium, with its conical protuberance on the upper surface, is similar, as is also the method of sexual reproduction by conjugation. However, there are certain differences. The sporangium is much smaller, opening by a single apical or lateral pore, and not by two lateral oppositely placed pores as is characteristic for *Rhizophidium columnaris*. Also in *Chytridium Spirotaenia* the male conjugation tube swells at its point of contact with the female, and the outer layer of the resting spore wall is densely covered with broad, solid, refractive, ray-like protuberances, giving it an irregular outline (Text-fig. 1 *b*, *c*). In view of these differences it is suggested that these two organisms cannot be recognized as identical, and a new species is erected, *Rhizophidium columnaris*, taking its name from the columnar bands of thickening on the resting spore wall.

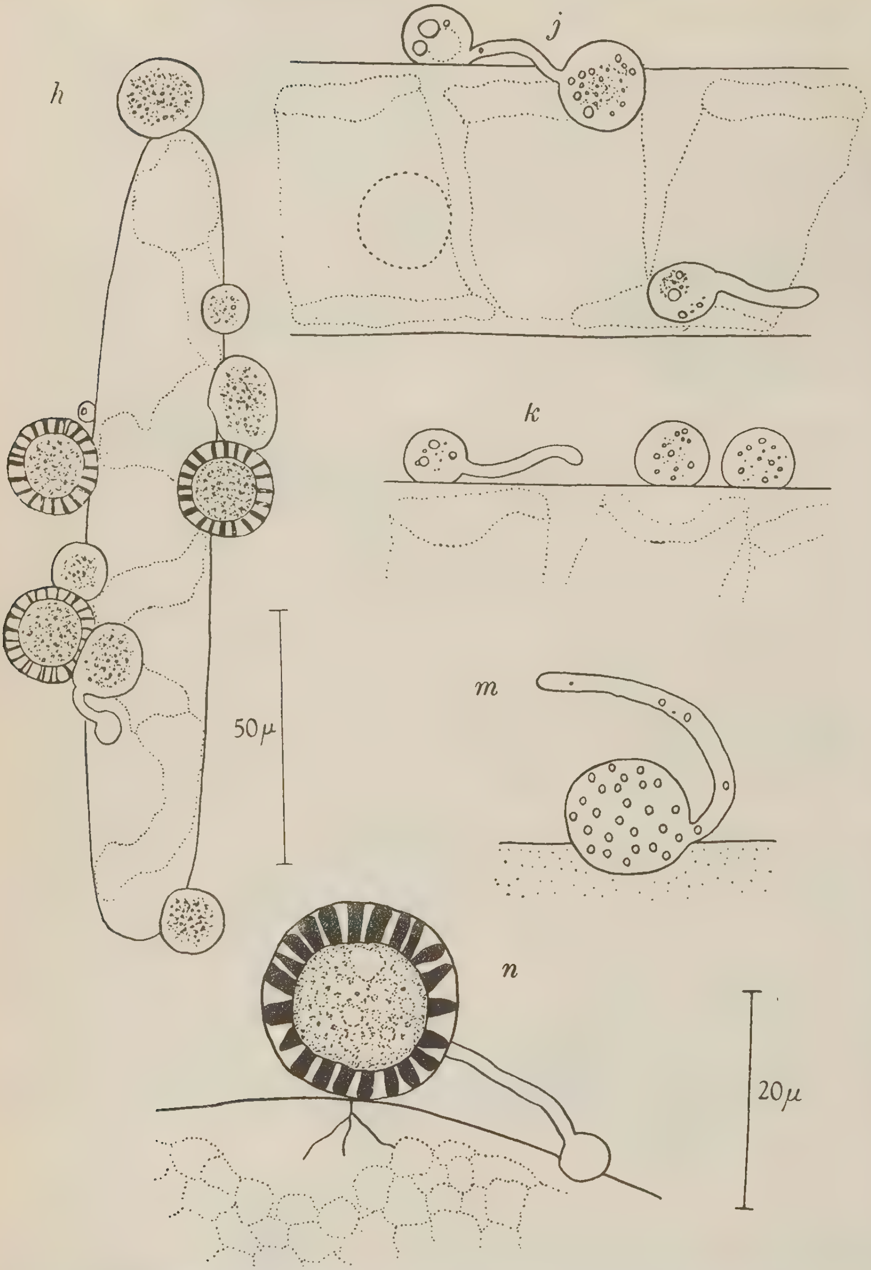
In *R. columnaris*, as in *Zygorhizidium*, certain aspects of its sexuality remain puzzling. Both Scherffel and Löwenthal describe dwarf thalli of *Z. Willei*, upon which a conjugation tube had formed, functioning as zoosporangia. In *Rhizophidium columnaris* one such specimen was observed (Text-fig. 4*m*). However, although the oil globules of the zoospores were delimited their actual liberation was not seen. This possibly gives further support to the suggestion, that the subsequent nature of the thallus produced from the zoospores in *Zygorhizidium* is determined by environmental conditions, and not due to inherent differences produced in the swarmers on germination of the resting spore.

Rhizophidium columnaris n.sp.

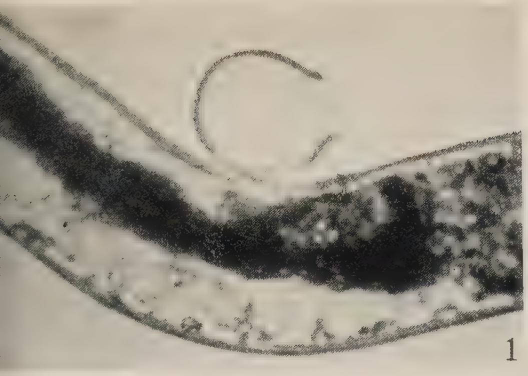
Thallus monocentric, epibiotic. Sporangium broadly ovoid, with its longer axis parallel to the host wall; wall smooth, colourless with a conical protuberance on its upper surface; large sporangia 25–63 μ broad × 16–27 μ high; dwarf sporangia 8–15 μ broad × 9–13 μ high; dehiscing by two broad, lateral, oppositely placed pores, very rarely one apical pore. Zoospores spherical, 2·6 μ in diameter, uniflagellate, with a conspicuous anterior oil globule and a darker area laterally; emerging singly, movement



Text-fig. 3. *Rhizophidium columnaris* n.sp. *a*, encysted zoospores. *b*, young zoosporangium with centralized highly refractive contents; rhizoids not visible. *c*, young zoosporangium with well-developed rhizoids, the conical protuberance is visible on the upper surface. *d*, mature sporangium, oil globules of the zoospores delimited. *e*, dehisced sporangium with zoospores. *f*, two empty sporangia and a mature dwarf sporangium. *g*, various stages in development of sporangia and resting spores. (All ×660, except *b*, ×1400.)



Text-fig. 4. *Rhizophidium columnaris* n.sp. *h*, stages in development of zoosporangia and resting spores. *j*, male thalli with developing conjugation tubes, one having fused with a female thallus. *k*, a male thallus, its conjugation tube approaching two female thalli. *m*, male thallus apparently functioning as a zoosporangium. *n*, mature resting spore showing highly refractive thickening bands of the wall, which are not as distinct from the rest of the wall as is suggested by the diagrammatic representation of the solid black against a white background. (All × 1400, except *h*, × 660.)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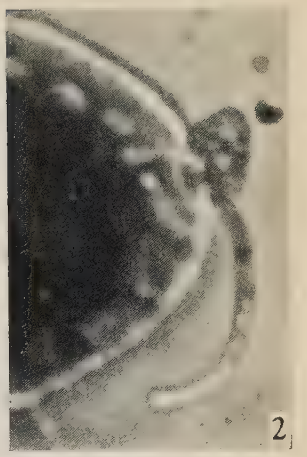
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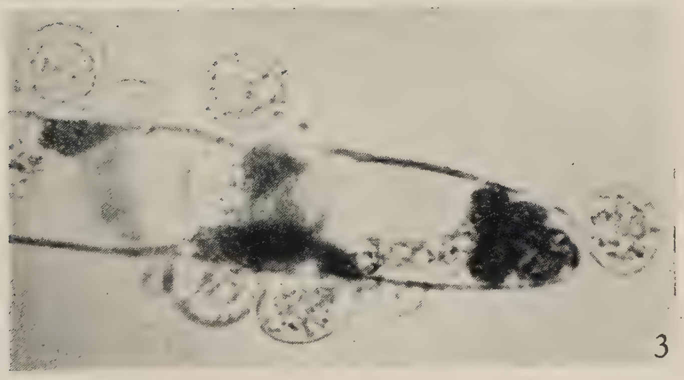
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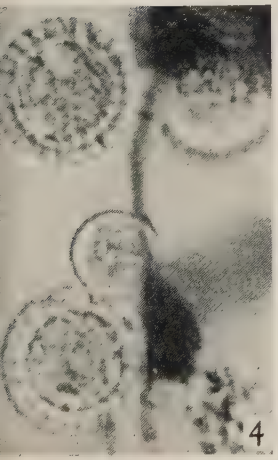
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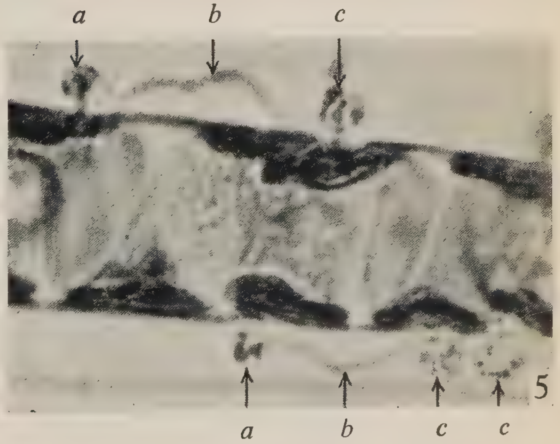

5

even gliding. Rhizoidal system branched arising from an indistinct main axis. Resting spore sexually formed, epibiotic, spherical, 10–20 μ, wall up to 3 μ thick, colourless, smooth, with columnar bands of refractive material; central contents granular, germination not observed. Male thallus epibiotic, spherical, connected to the female by a narrow cylindrical conjugation tube 2 μ diameter and up to 38 μ long.

Parasitic on *Spirotaenia condensata* Brèb., Clay Pond, Wray Castle, England.

Rhizophidium columnaris sp.nov.

Thallus monocentricus, epibioticus. Sporangium late ovoideum, decumbens, hyalinum, laeve, episporio una extremitate papillato; sporangia majora 25–63 × 16–27 μ, sporangia nana 8–15 × 9–13 μ, a duobus poris lateralibus oppositis vel rare a singulo poro apicale dehiscentia. Zoosporae sphaericae, 2·6 μ diam., uniflagellatae, guttula anteriore distincta parteque obscuriore laterale praeditae, singulatim emergentes. Rhizoidea ramosa ex axe parum claro oriunda. Sporae perdurantes epibioticae, sphaericae, 10–20 μ diam., intus granulosae, episporio ad 3 μ crasso, hyalino, laeve, interne lineis radiantibus refringentibus praedito; germinatio non visa. Thallus masculinus epibioticus, sphaericus, tubulo anguste 2 μ diam. et ad 38 μ longo cum femine conjunctus.

Hab. in *Spirotaenia condensata* Brèb. parasiticus, Clay Pond, Wray Castle, England.

My thanks are due to the Director of the Freshwater Biological Association for the use of a laboratory in which this work was done, to Miss E. M. Wakefield for the Latin diagnosis and especially to Prof. C. T. Ingold for his helpful criticism.

REFERENCES

DOMJÁN, A. (1936). 'Vízigombás'-Adatok Szeged és Tihany Vidékéröl ('Wasserpilz'-daten aus der Umgebung von Szeged und Tihany). *Folia cryptogam.* II (1), 8–59.

GEITLER, L. (1942). Eine neue Chytridiale, *Zygorhizidium verrucosum*, n.sp., und ihre Wirkung auf die Wirtszellen. *Arch. Protistenk.* XCVI (1), 110–18.

LÖWENTHAL, W. (1905). Weitere Untersuchungen an Chytridiaceen. *Arch. Protistenk.* V, 221–39.

SCHERFFEL, A. (1925). Zur Sexualität der Chytridineen (Der 'Beiträge zur Kenntnis der Chytridineen', Teil I). *Arch. Protistenk.* LIII, 1–58.

SPARROW, F. K. (1943). *Aquatic Phycomycetes.* Ann Arbor, U.S.A.: University of Michigan Press.

EXPLANATION OF PLATE XI

Zygorhizidium Willei Löwenthal and *Rhizophidium columnaris* n.sp.

Fig. 1. Empty sporangium of *Zygorhizidium Willei* with a broad lateral dehiscence pore. A slight curvature of the *Mougeotia* cell brought about by the fungus is visible. × 1030.

Fig. 2. *Zygorhizidium Willei*, male thallus with its conjugation tube. × 960.

Fig. 3. Part of a *Spirotaenia* cell showing zoosporangia and resting spores of *Rhizophidium columnaris*. × 820.

Fig. 4. A portion of fig. 3 more highly magnified showing two resting spores and a young zoosporangium. The columnar bands of thickening material are visible in the wall of the upper resting spore. × 1287.

Fig. 5. Part of a *Spirotaenia* cell with male and female thalli of *Rhizophidium columnaris*. (*a*) male thalli, (*b*) conjugation tube, (*c*) female thalli. × 1200.

(*Accepted for publication* 14 *January* 1947)

ANTI-FUNGAL SUBSTANCES FROM MOULDS

PART II. THE EFFECT OF PATULIN ON THE GROWTH OF VARIOUS PLANT PARASITES

By GEORGE SMITH, *London School of Hygiene and Tropical Medicine*

Van Luijk (1938), in a paper dealing with antagonism between soil fungi, showed that, when *Penicillium expansum* (Link.) Thom. is grown on Knop's solution containing 4% sucrose, the culture filtrate has very marked powers of inhibiting the growth, *in vitro*, of *Pythium de Baryanum*. In addition, good results were obtained in suppressing the attack of the *Pythium* on lucerne seedlings by treating infected soil with the active filtrate. The substance responsible for the inhibitory effect was not, however, isolated and characterized.

Anslow, Raistrick and Smith (1943) found that patulin, previously isolated as a metabolic product of *Penicillium patulum* Bainier, is produced also, though in smaller yields, by various strains of *P. expansum* isolated from rotting fruits. It was demonstrated that patulin completely inhibits the growth of a number of species of *Pythium* at a concentration of 1:400,000, and that the fungistatic activity of culture filtrates from *Penicillium expansum* or *P. patulum* is in good agreement with their content of patulin, rendering it highly probable that van Luijk's active principle was indeed patulin.

It is now shown that patulin inhibits the growth of a number of other parasitic fungi, albeit at higher concentrations than that required to suppress the growth of species of *Pythium*. *Rhizoctonia Solani* Kühn is completely inhibited by patulin at a concentration of 1 : 10,000, *Sclerotium Rolfsii* Saccardo at 1 : 20,000, *Verticillium albo-atrum* Reinke & Berth. at 1 : 10,000, *Helminthosporium monoceras* Drechsler at 1 : 20,000, *H. gramineum* Rabenhorst at 1 : 20,000, *H. sativum* Pamm., King & Bakke at 1 : 5000, *Ceratostomella Pini* Münch. at 1 : 20,000, whilst *Botrytis cinerea* Pers. ex Fries is more resistant, being only partially inhibited at a concentration of 1 : 5000. It was also considered of interest to test the effect of patulin on *Trichoderma viride* Pers. ex Fries, a mould which itself produces anti-fungal substances (Weindling, 1932; Brian & McGowan, 1945). This species, like *Botrytis cinerea*, is only partially inhibited at a concentration of 1:5000. At lower concentrations the effect of patulin is stimulatory, giving more abundant fructification and enhanced production of pigment.

EXPERIMENTAL

Cultures. Typical strains of *Rhizoctonia Solani* (L.S.H.T.M. Catalogue no. BB192) and *Sclerotium Rolfsii* (BB191) were obtained from the National Collection of Type Cultures. Two strains of *Verticillium albo-atrum* were received from the East Malling Research Station, where they had been isolated from diseased hops, one (X 23) from a 'progressive' outbreak of the disease, the other (X 24) from a typical 'fluctuating' infection. The culture of *Helminthosporium monoceras* (Ag 117) was obtained in 1932 from C.B.S., Baarn. This species of the genus was included because it grows well, and regularly produces abundant conidia, on a variety of culture media. The strain of *H. gramineum* (Ag 106) was obtained from Prof. F. T. Brooks in 1925 and does not produce conidia in culture. *H. sativum* (Ag 105) was also obtained from Prof. Brooks, in 1932. Cultures on a variety of media produce conidia only tardily and sparingly. The strain of *Botrytis cinerea* (BB9A) was isolated in this laboratory from a wilted *Calceolaria* seedling. The culture of *Trichoderma viride* was isolated in 1927 from cotton yarn. It is a fast-growing strain and produces abundant yellow pigment on potato dextrose agar or wort-agar. *Ceratostomella Pini* was obtained from Dr W. P. K. Findlay.

METHOD OF TEST

The method described by van Luijk (1938), and used in this laboratory for the experiments with species of *Pythium*, is not suitable for testing organisms which grow only with difficulty when inoculated at the bottom of a tube of liquid medium. The method adopted was to make solutions of patulin in melted agar culture medium, pour into Petri dishes, and to sow each dish at a single central point with the fungus to be tested. In every series two or three dishes, containing the agar medium without patulin, were used as controls. The period of incubation varied with the fungus under test, but it was always at least as long as the time required for the control plates to be completely covered with typical growth.

As a preliminary, the action of patulin on *Pythium ultimum* was tested in this way, in order to find whether the two methods give comparable results.

The agar solutions of patulin were a series of twofold dilutions from 1 : 80,000 to 1 : 1,280,000. A number of solutions were made of patulin in Czapek-Dox solution, the concentrations being fifteen times the final concentrations required. These solutions were sterilized by steaming for fifteen minutes. Each final dilution was made by adding 1 ml. of the appropriate solution, with aseptic precautions, to a tube containing 14 ml. of melted Czapek agar at 45° C. The contents of the tube were well mixed and poured into a Petri dish. Dilutions were made in triplicate, each dish being inoculated with a fragment of mycelium from a young, vigorously growing culture of *Pythium ultimum*. The dishes were incubated at 24° C. for six days. Growth was completely inhibited at 1 : 320,000 and partially inhibited at 1 : 640,000. The activity of patulin, as shown by this method of test, is therefore of the same order as that shown by the earlier method, using liquid culture media.

RESULTS

Rhizoctonia Solani was tested in the same way as *Pythium*, but using a dilution series starting at 1 : 5000. The results were somewhat erratic. In one series there was complete suppression of growth on all plates at

1 : 10,000, two out of three plates at both 1 : 20,000 and 1 : 40,000 showed no growth after thirteen days' incubation, and there was definite retardation of growth at 1 : 80,000 and 1 : 160,000. In a second series there was again complete inhibition at 1 : 10,000, and on one plate at 1 : 20,000, but with little evidence of partial inhibition at higher dilutions.

Sclerotium Rolfsii. A series of tests using Czapek agar as the basal medium gave irregular results, but growth on the control plates, containing no patulin, was also erratic. The fungus grows much better on potato dextrose agar, and this medium was selected for a second series of tests. It was found, however, that solutions of patulin in potato extract turn brownish on heating. The *p*H of the extract is approximately 6, at which reaction patulin is not heat-stable. In order to avoid destruction of the substance during sterilization, the primary dilutions were made in potato extract containing dextrose, previously adjusted to *p*H 4·5 by addition of hydrochloric acid. In making the final dilutions in potato dextrose agar, the *p*H of the latter was not adjusted, since the temperature did not exceed 45° C. and, after addition of the patulin solution, the agar was maintained at this temperature for only a few seconds before being poured into a cold Petri dish. The dilution series used was 1 : 5000 to 1 : 80,000 in twofold steps. Plates were sown with fragments of mycelium from a young culture of *S. Rolfsii*, since the sclerotia are germinated only with difficulty in the laboratory.

There was complete inhibition at 1 : 20,000, with no sign of partial inhibition at higher dilutions. This clean line between complete inhibition at 1 : 20,000 and normal growth, with production of abundant sclerotia, at 1 : 40,000, is in sharp contrast to the reaction of *Verticillium albo-atrum.*

Verticillium albo-atrum was tested on Czapek agar, the dilution series being 1 : 5000, 1 : 10,000 and then twofold dilutions from 1 : 25,000 to 1 : 200,000. Both strains, X 23 and X 24, gave similar results, and with each there was very little variation between the three plates used for each dilution. Inhibition was complete at 1 : 10,000 and there was progressively more abundant growth from 1 : 25,000 to 1 : 200,000. Even at the highest dilution, however, there was appreciable retardation of growth, as compared with the controls.

Helminthosporium monoceras. Dilutions were made in Czapek agar, the series being 1 : 5000 to 1 : 160,000 in twofold steps. There was complete inhibition of growth at 1 : 10,000, and no visible growth at 1 : 20,000 after sixteen days' incubation. At the latter dilution microscopic examination of the plates after three days showed that a high percentage of the conidia used as inoculum had germinated. Further incubation, however, resulted in no further growth, the germ-tubes remaining short, unbranched, and abnormal in appearance. There was definite retardation of growth up to 1 : 80,000.

Helminthosporium gramineum. Potato dextrose agar was used for the tests, the primary dilutions being made with potato extract adjusted to *p*H 4·5, as for *Sclerotium Rolfsii.* The fungus was completely inhibited at a concentration of 1 : 20,000 and growth was retarded even at the highest dilution used, 1 : 160,000. In the early stages of growth all colonies showed the typical purplish pigmentation, but after about ten days the plates at dilutions 1 : 40,000 and 1 : 80,000 showed only small central areas of pigment, whilst in the controls and the 1 : 160,000 plates pigmentation was general.

Helminthosporium sativum. This was tested on potato agar, at dilutions from 1 : 5000 to 1 : 160,000. Growth was completely inhibited at 1 : 5000 and there was partial inhibition at all higher dilutions. The colonies on different concentrations of patulin showed very little variation as regards texture and tendency to sporulation.

Ceratostomella Pini. This species also was tested on potato agar, as this has been found to be most favourable for production of perithecia. There was complete inhibition at 1:20,000 and retardation of growth up to 1:80,000. At 1:40,000 there was no growth after three days' incubation, although in this period the colonies on the control plates had reached 3·5 cm. in diameter, and even after seventeen days colonies remained thin and pale coloured, without any suggestion of perithecia.

Botrytis cinerea was tested in exactly the same way as *Verticillium albo-atrum.* At a concentration of patulin of 1 : 10,000 there was no apparent effect on the growth of the fungus, and at 1 : 5000 there was only slight retardation of growth.

Trichoderma viride. Czapek agar was used for dilutions from 1 : 5000 to 1 : 160,000. The control plates and those containing dilutions 1 : 10,000 upwards were completely covered by the mould in six days. At this stage the 1 : 5000 plates had colonies about 5 cm. in diameter, but after nine days even these plates were completely covered. The agar in the controls and the 1 : 5000 dilution plates remained colourless for several days and showed only a faint yellow colour after nine days' incubation. At all other dilutions the agar was definitely pigmented after three days and rapidly turned deep yellow. There is no doubt that, on Czapek agar, patulin in low concentrations stimulates pigment production.

REFERENCES

ANSLOW, W. K., RAISTRICK, H. & SMITH, G. (1943). Anti-fungal substances from moulds, Part I. Patulin (anhydro-3-hydroxymethylene-tetrahydro-1:4-pyrone-2-carboxylic acid), a metabolic product of *Penicillium patulum* Bainier and *Penicillium expansum* (Link) Thom. *J. Soc. Chem. Ind., Lond.*, LXII, 236–8.

BRIAN, P. W. & McGOWAN, J. C. (1945). Viridin: a highly fungistatic substance produced by *Trichoderma viride. Nature, Lond.*, CLVI, 144–5.

VAN LUIJK, A. (1938). Antagonism between various micro-organisms and different species of the genus *Pythium* parasitizing upon grasses and lucerne. *Med. Phytopath. Lab. 'Willie Commelin Scholten'*, XIV, 45–83.

WEINDLING, R. (1932). *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopath.* XXII, 837–45.

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THE MEASUREMENT OF POTATO BLIGHT

The method of measuring Potato Blight on the foliage proposed by a Sub-Committee of the Society's Plant Pathology Committee (Moore, 1943, p. 34) has now been tested over several seasons by a number of observers, and its practical usefulness has been satisfactorily established. Briefly, the method is to assess the percentage of leaf area destroyed by Blight by eye judgement based on the following Key, which is now presented in its finally revised form.

KEY

Percentage (D.M.)	Description
0	Not seen on field
0·1	Only a few plants affected here and there; up to one or two spots in 12 yd. radius
1	Up to ten spots per plant, or general light spotting
5	About fifty spots per plant or up to one leaflet in ten attacked
25	Nearly every leaflet with lesions, plants still retaining normal form: field may smell of blight, but looks green although every plant affected
50	Every plant affected and about one-half of leaf area destroyed by blight: field looks green flecked with brown
75	About three-quarters of leaf area destroyed by blight: field looks neither predominantly brown nor green. In some varieties the youngest leaves escape infection so that green is more conspicuous than in varieties like King Edward, which commonly shows severe shoot infection
95	Only a few leaves left green, but stems green
100	All leaves dead, stems dead or dying

In the earlier stages of a blight epidemic parts of the field sometimes show more advanced decay than the rest and this is often associated with the primary foci of the disease. Records may then be made as, say 1 + pf25, where pf25 means 25 % in the area of the primary foci.

Previous methods of blight measurement have adopted arbitrary scales of blight amounts. The present method has the advantage, that the figure given in each case, is a measure within fairly accurate limits of the actual percentage of leaf area destroyed. The error at the bottom of the scale will probably be less in magnitude than in the region of 50 and 75 %, but great accuracy is not really essential in this type of work, particularly when several observations are made at different dates through the season.

It should be emphasized that the method of Potato Blight measurement here outlined applies only to the disease on the *foliage*. For disease in the *tubers* a quite different method should be adopted. For most purposes the percentage of infected tubers is what is required and variations in the amount of disease on individual tubers are rarely of importance. Whether the tubers are in the soil or in the clamps the percentage of infection is easily obtained by the counting of samples, and if the samples are sufficient in number and taken at random, accurate estimations for the whole crop can be made.

REFERENCE

Moore, W. C. (1943). The measurement of plant diseases in the field. *Trans. Brit. mycol. Soc.* xxvi, 28–35.

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SPRING FORAY, 1946

WHEATFEN BROAD, NORFOLK

A visit to Wheatfen Broad, Surlingham, Norfolk, from Friday to Monday, 24–27 May 1946, marked the occasion of the first post-war Spring Foray.

This visit was made possible by the kindness of Mr E. A. Ellis who, in addition to entertaining a party of members at his home at Wheatfen, placed his workroom and mycological library at the disposal of the visitors and gave unstintingly of his unrivalled knowledge of the locality. The Society is greatly indebted to Mr and Mrs Ellis for all they contributed to the success of the Foray.

Wheatfen Broad is an area of some 150 acres of Yare Valley swamp situated about seven miles from Norwich, to the north of Rockland Broad, and bounded on the east by the river Yare and on the slightly higher ground to the south-west by Surlingham Wood. The main estate consists of a series of 'marshes' intersected by several miles of shallow streams connecting a chain of small broads navigable to a punt. Tidal communication with the Yare is responsible for a regular circulation of water and the normal rise and fall of 8 in. in the water level. The characteristic features of the vegetation are reed swamp, *Glyceria* marsh, *Cladium* fen, and sallow-ash carr. This area, which is of great biological interest, and which will it is hoped be permanently preserved, has been studied intensively for the past ten years by a group of naturalists who have published their results in a series of papers in the *Transactions of the Norfolk and Norwich Naturalists Society* (see Ellis, XIII, 422–51, 1934, for a general account of the vegetation and XV, 191–219, 1941, for a list of micro-fungi by the same author).

Most of the twenty-five members and friends attending the Foray found themselves on unfamiliar ground and all found much of interest. The main objective was micro-fungi and the collections made represented more than a hundred species belonging to diverse groups. All the finds have been entered on the card index kept by Mr Ellis with a view to the eventual publication of a critical account of the fungi of Wheatfen so that here it has only been thought necessary to supplement comments on some of the more interesting records with a list of fungi found in association with certain marsh plants. Specimens of most of the finds have been deposited in the herbaria of Mr E. A. Ellis, the Imperial Mycological Institute, or the Royal Botanic Gardens, Kew.

Helicosporium phragmitis, which is not included in Wakefield and Bisby's list of British Hyphomycetes, was found in abundance on decaying stems of *Glyceria maxima*. These, according to A. E. Ellis, are its characteristic habitat in the Yare Valley but it has also been recorded on *Phragmites*, *Phalaris arundinacea*, and *Calamagrostis arundinacea*. *Clasterosporium canicinum* on *Carex* attracted attention, for this fungus (originally described by Schweinitz in North America) appears to have been recorded for Europe only from

Norfolk. Among other Hyphomycetes, *Myrothecium inundatum* Fr. on an old sporophore of *Russula adusta* and *Pachybasium hamatum* (Bon.) Sacc. found growing with *Rhytisma acerinum* on sycamore leaves may be mentioned, while Prof. C. T. Ingold listed seven aquatic species on dead leaves in the tidal ditches. The resemblance of *Mitrula sclerotipus* growing from sclerotia on blackened remains of meadowsweet leaves to species placed in *Verpatina* Whetzel & Drayton, was noted and *Pachydiscia marchantiae* (Berk.) Boud. was found on the liverwort *Conocephalum*. The most noteworthy rust recorded was *Puccinia persistens* Plowr., forming abundant aecia on *Thalictrum flavum*. Larger basidiomycetes were infrequent but Miss Wakefield added six species of *Corticium*, including *C. niveocremeum* and *C. roseocremeum*, and nine of *Peniophora*, including *P. candida* Lyman (syn. *P. aegerita*) with the *Aegerita* stage, *P. leprosa*, and *P. pubera* to the list. There have been many records of entomogenous fungi from Wheatfen. During the Foray *Gibellula aranearum* (Schwein.) Syd. on spiders and *Isaria farinosa* (Holmsk.) Fr. on moth pupae were found.

Acknowledgement must be made to Mr M. B. Ellis, Dr R. W. G. Dennis, Mr S. J. Hughes, and Mr E. W. Mason for making critical determinations.

List of fungi associated with certain marsh plants at Wheatfen Broad

Caltha palustris. Ramularia calthae (Erikss.) Lindr.
Carex. Marasmius menieri (on *C. riparia*), Arthrinium sporophlaeum Fr., Clasterosporium caricinum Schw., Gonatosporium puccinioides (Fr.) Corda, Volutella melaloma Berk. & Br.
Cladium mariscus. Pistillaria aculeata, Tetraploa aristata Berk. & Br.
Equisetum palustre. Stamnaria persooni (Fr.) Fuckel.
Filipendula ulmaria. Mitrula sclerotipus Boud.
Galium aparine. Peronospora calotheca de Bary.
Glyceria maxima. Gibberella zeae (Schw.) Petch, Coprinus (? urticaecola), Dematium hispidulum Fr., Helicosporium phragmitis Höhnel.
Myosotis. Synchytrium aureum Schroet., Erysiphe cichoracearum DC.
Myrica gale. Ovularia destructiva (Phill. & Plowr.) Massee.
Peucedanum palustre. Plasmopara nivea (Ung.) Schroet. (also on *Aegopodium* and *Angelica*).
Phragmites communis. Dasyclypha controversa (Cooke) Rehm, Pyrenopeziza arundinacea (DC.) Boud., Lophodermium arundinaceum (Fr.) Chev., Napicladium arundinaceum (Corda) Sacc.
Poa trivialis. Passalora graminis (Fuckel) Höhnel.
Salix. Bertia moriformis (Fr.) de Not., Calyculosphaeria tristis (Fuckel) Fitzpat., Chaetosphaeria phaeostroma (Dur. & Mont.) Fuckel, Diaporthe eres Nits., Eutypa flavovirens (Fr.) Tul., Gloniopsis levantica Rehm, Hypoxylon rubiginosum (Fr.) Fr., Lophiostoma salicum (Fabr.) Sacc., Nitschkia cupularis (Fr.) Karst., Rosellinia aquila (Fr.) de Not., Pholiota erinacea (Fr.) Quel., Coniothecium amentacearum Corda.

REVIEW

Flax Diseases. By R. McKay. (Flax Development Board, Ltd.; 2 Kildare Place, Dublin, 1947.) 55 pp., 52 figs. Price 5*s.*

At the beginning of the War flax jumped into prominence as an important crop: and in England and Wales alone the area devoted to it increased rapidly from about 4500 acres in 1939 to over 60,000 acres in 1944. Despite the wise precaution of ensuring that all flax seed was suitably disinfected with Nomersan before sowing, some anxiety was not unnaturally felt about the possible development and spread of disease in the crop, and this in turn revealed a gap in plant disease literature, for no general account of flax diseases was available to farmer or adviser. This gap has now been filled very adequately by Prof. McKay's informative booklet, which provides all those interested in flax cultivation with a concise account of its pathology and a means whereby all the significant diseases and pests of the crop can be recognized in the field. The text is a happy blend which satisfies the plant pathologist without diminishing the value of the bulletin as a guide for the interested factory fieldsman and grower. Well-selected references are given after the description of each disease, though a few recent ones that might be expected in a publication appearing in 1947 are missing. There is a glossary of technical terms, and indexes to common and scientific names as well as to authors. The bulletin is very attractively designed and profusely illustrated: it would be interesting, however, to see the effect of applying colour work to this rather difficult photographic subject. Mycologists may well be puzzled by the invariable use of brackets for the authorities of the fungus names.

W. C. Moore

EDITORIAL NOTE

It is intended, as soon as circumstances allow, to issue the Proceedings of the Jubilee Celebrations of the British Mycological Society as Volume XXX of the *Transactions*: this volume will be sent to all who were members on 30 June 1947. In accordance with this intention, the present volume is numbered XXXI.

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